Land-use effects on soil quality of agricultural systems in the Central Andes of Bolivia

Thesis presented in partial fulfillment of the requirements for the degree of Doctor of Sciences

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Summary

Increasing demands for food of the growing world population has led to agricultural intensification and rapid changes in land use worldwide. Resulting modifications of soil characteristics could have cascading effects on soil functioning and the delivery of ecosystem services (e.g. food production). Maintenance of soil quality, the capacity of a soil to function to sustain biological productivity while maintaining environmental quality, is thus of central importance in order to ensure long-term provision of food, and its evaluation allows to understand the soil responses and to predict potential consequences of land-use changes.

Over the last decades, agricultural systems of the Central Andean region of Bolivia have experienced rapid changes of land use and management practices such as shortening of fallow periods, use of chicken manure fertilization to improve soil fertility, and the plantation of *Eucalyptus globulus* L. on arable soils. So far, the effects of these changes on soil quality have not been assessed. Improving agricultural production while contributing to sustainability of these ecosystems requires soil quality evaluations under the current land uses. This thesis evaluated the effect of fields cultivated with potato, fallow fields (2-6 years old), eucalyptus plantations, and the use of chicken manure for fertilization on soil quality in agricultural ecosystems of the Central Andes. The combined responses of soil physico-chemical properties, soil aggregates, microbial processes, and their interactions were evaluated as indicators of soil quality.

Short-term fallowing did not contribute to soil fertility restoration or soil structure improvement, indicating a neutral effect on soil quality compared to fields cultivated with potato. However, fallowing soils lead to reduced net nitrogen transformation rates and lower abundance and activity of ammonia oxidizing bacteria, suggesting sensitivity of these processes to land-use change.

Fertilization with chicken manure improved soil quality by increasing labile carbon and nitrogen fractions and microbial activity. Manure also promoted seed germination and plant growth, however, immature manure caused root damage on *Avena sativa* L. seedlings. Turning manure piles accelerated the composting process and reduced root damage. It is recommended to compost manure for 100 days and to conduct turning events to reduce the risk of manure phytotoxicity.

The plantation of eucalyptus promoted organic matter accumulation, and increased the total amount of carbon in different fractions. Soil structure was also improved and contributed to carbon stabilization. These changes are commonly associated with an improvement of soil quality for cultivation, but increased exchangeable Al and potential allelopathic interactions reduced biological soil quality compared to cultivated fields (reduced metabolic potential of soil bacteria, N transformation rates, and increased microbial energy maintenance demands) The plantation of eucalyptus should be avoided in agricultural areas, and careful land-use planning should guide its plantation to avoid negative effects on crops.

Under the three land uses, ammonia-oxidizing archaea were dominant nitrifying organisms in terms of abundance and activity, but the contribution of ammonia-oxidizing bacteria to nitrification significantly increased with pH. We have also demonstrated that the activity of ammonia oxidizers should not be inferred from *amoA* gene abundances alone, but that their nitrification rates should be measured separately to determine their role in the environment. Labile carbon fractions (hot water extractable carbon and microbial biomass carbon) were strongly associated to microbial processes and were sensitive to land-use changes. Moreover, increases in microbial activity (respiration potential and net nitrogen mineralization) were associated to aggregate formation and stability. Labile carbon fractions and microbial processes should be considered for monitoring soil quality in response to land-use change and agricultural practices.

This study demonstrated that the short fallow periods did not contribute to soil quality improvement for cultivation compared to fields cultivated with potato. The use of composted chicken manure for fertilizing soils improved to soil quality for agricultural production but the use of immature manure should be avoided to reduce the risk of phytotoxic effects. The introduction of eucalyptus to the Andean agricultural systems reduced soil quality for cultivation compared to fields cultivated with potato. Labile carbon fractions and microbial processes stand out as sensitive and informative soil quality indicators of the early changes occurring in these agricultural systems following land-use change.

Keywords: Soil microbial activity, soil aggregates, chicken manure, S*olanum tuberosum*, fallow, *Eucalyptus globulus.*

Résumé

La demande croissante de nourriture due à l'augmentation de la population mondiale a conduit à une intensification des pratiques agricoles et à des changements rapides dans l'utilisation des terres dans le monde entier. Les modifications des caractéristiques du sol résultantes peuvent avoir des effets en cascade sur son fonctionnement et sur la fourniture de services écosystémiques (par exemple, la production alimentaire). Le maintien de la qualité du sol, sa capacité à fonctionner pour soutenir la productivité végétale tout en maintenant la qualité de l'environnement, est donc d'une importance capitale pour assurer un approvisionnement alimentaire à long terme, et son évaluation permettrait de prédire de potentielles conséquences des changements d'affectation des terres.

Au cours des dernières décennies, les systèmes agricoles de la région des Andes centrales de la Bolivie ont connu des changements rapides d'utilisation des terres et de pratiques de gestion, tels que le raccourcissement des périodes de jachère, l'introduction d'*Eucalyptus globulus* L. sur les sols arables et la fertilisation avec du fumier de poulet pour améliorer la fertilité des sols. Jusqu'à présent, les effets de ces changements sur la qualité des sols n'ont pas été évalués. Pour l'amélioration de la production agricole et la durabilité de ces écosystèmes, des évaluations de la qualité des sols dans le cadre des utilisations actuelles des terres sont nécessaires. Cette thèse a évalué l'effet de la culture de pomme de terre, de la mise en jachère (2-6 ans), de la culture d'eucalyptus et de l'utilisation de fumier de poulet comme fertilisant sur la qualité des sols dans les écosystèmes agricoles des Andes centrales. Les réponses combinées des fractions de carbone du sol, des agrégats, des processus microbiens et de leurs interactions ont été évaluées en tant qu'indicateurs de la qualité du sol.

La jachère à court terme n'a pas contribué à la restauration de la fertilité du sol ou à l'amélioration de la structure du sol, indiquant un effet neutre sur la qualité du sol par rapport aux champs cultivés avec des pommes de terre. Cependant, la mise en jachère entraîne une réduction des transformations nettes de l'azote et une diminution de l'abondance et de l'activité des bactéries oxydant l'ammoniac, ce qui suggère une sensibilité de ces processus au changement d'utilisation des terres.

La fertilisation avec du fumier de poulet a améliorée la qualité du sol, en augmentant les fractions labiles de carbone et d'azote et l'activité microbienne. Le fumier a également favorisé la germination des graines et la croissance des plantes ; cependant le fumier immature a causé des dommages aux racines des semis d'*Avena sativa* L. Le retournement des tas de fumier a accéléré ce processus de compostage et a réduit les dommages aux racines. Il est donc recommandé de composter le fumier pendant 100 jours et de procéder à des retournements des tas pour réduire le risque de phytotoxicité du fumier.

La plantation d'eucalyptus a favorisé l'accumulation de matière organique et augmenté la quantité totale de carbone dans différentes fractions. La structure du sol a également été améliorée et a contribué à la stabilisation du carbone. Ces changements sont généralement associés à une amélioration de la qualité du sol pour la culture, mais l'augmentation de l'aluminium échangeable et des interactions allélopathiques potentielles ont réduit la qualité

biologique du sol par rapport aux champs cultivés (potentiel métabolique des bactéries du sol et transformations nettes de l'azote réduits, augmentation de besoin en énergie de maintenance des micro-organismes). La plantation d'eucalyptus doit être évitée dans les zones agricoles, et sa plantation doit être accompagnée d'une planification pour éviter les effets négatifs sur les cultures.

Les archées oxydant l'ammoniac étaient des organismes nitrifiants dominants en termes d'abondance et d'activité pour les trois utilisations des terres étudiées, mais la contribution des bactéries oxydant l'ammoniac à la nitrification augmentait significativement avec l'augmentation du pH. Nous avons également démontré que l'activité des oxydants d'ammoniac ne doit pas être déduite de l'abondance des gènes *amoA* seule, mais que leur vitesse de nitrification doit être mesurée pour déterminer leur rôle dans l'environnement. Les fractions de carbone labiles (le carbone extractible à l'eau chaude et la biomasse microbienne) étaient fortement associées aux processus microbiens et sensibles aux changements d'utilisation des terres. L'augmentation de l'activité microbienne (potentiel de respiration et minéralisation nette de l'azote) a également été associée à la formation et à la stabilité des agrégats. Les fractions de carbone labiles et les processus microbiens devraient être pris en compte pour surveiller la qualité des sols en réponse aux changements d'utilisation des terres et aux pratiques agricoles.

Cette étude a démontré que les courtes périodes de jachère ne contribuaient pas à l'amélioration de la qualité des sols par rapport aux champs cultivés avec des pommes de terre. L'utilisation de fumier de poulet composté comme fertilisant a amélioré la qualité des sols agricoles, mais l'utilisation de fumier immature doit être évitée pour éviter le risque d'effets phytotoxiques. L'introduction de l'eucalyptus dans les systèmes agricoles andins a réduit la qualité du sol pour l'agriculture par rapport aux champs cultivés avec des pommes de terre. Les fractions de carbone labile et les processus microbiens se distinguent comme des indicateurs précoces, sensibles et informatifs de changements dans la qualité des sols dans ces systèmes agricoles à la suite d'un changement d'affectation des terres.

Mots clés: activité microbienne, agrégats du sol, fumier de poule, *Solanum tuberosum*, jachère, *Eucalyptus globulus*.

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List of Acronyms

Al_{DCB}	Dithionite extractable Aluminum
Al_{Ox}	Oxalate extractable Aluminum
AOA	Ammonia-oxidizing archaea
AOB	Ammonia-oxidizing bacteria
ATU	Allylthiourea
AWCD	Average well color development
C:N	Carbon to nitrogen ration
COIA	Co-inertia analyses
EBC	Exchangeable base cations
Fe_{DCB}	Dithionite extractable iron
Feox	Oxalate extractable iron
HWC	Hot water extractable carbon
HWN	Hot water extractable nitrogen
LMM	Linear mixed models
MBC	Microbial biomass carbon
MBN	Microbial biomass nitrogen
MBP	Microbial biomass phosphorus
Mn_{DCB}	Dithionite extractable Manganese
Mn_{Ox}	Oxalate extractable Manganese
MWD	Mean weight diameter
Nmin	Net nitrogen mineralization
PCA	Principal component analyses
PNR	Potential nitrification rate
qCO2	Metabolic quotient
qmic	Microbial quotient
qPCR	Quantitative polymerase chain reaction
RDA	Redundancy analyses
SOM	Soil organic matter
TOC	Total soil organic carbon
TN	Total soil nitrogen
WHC	Water holding capacity
WSA	Water stable aggregates
WSC	Water soluble carbon
WSN	Water soluble nitrogen

Scientific communications from the PhD research

Journal articles

Coca-Salazar A., Richaume A., Florio A., Carnol M. 2021. Response of ammonia-oxidizing bacteria and archaea abundance and activity to land use changes in agricultural systems of the Central Andes. 102: 1-8. European Journal of Soil Biology. DOI: 10.1016/j.ejsobi.2020.103263

Coca-Salazar A., Cornelis J.T., Carnol M. 2021. Soil properties and microbial processes in response to land-use change in agricultural highlands of the Central Andes. European Journal of Soil Science. DOI: 10.1111/ejss.13110

Conference abstracts

Coca-Salazar A., Cornelis J.T., Carnol M. 2017. Actividad microbiana en suelos agrícolas, en descanso y con uso forestal en una zona productora de papa, Cochabamba-Bolivia. VIII Congreso boliviano de la ciencia de suelo. Santa Cruz, Bolivia.

Coca-Salazar A., Richaume A., Carnol M. 2019. Activity and abundance of ammonia oxidizing bacteria and archaea in Bolivian soils. 4th Thünen Symposium on soil metagenomics. Braunschweig, Germany.

General context

Over the past century, agricultural practices have intensified and led to rapid land-use changes in order to meet the demands for food of an increasing world population (Godfray et al., 2010; Leisinger et al., 2002). Land-use changes include modifications of vegetation cover, biological diversity, and soil management practices, which may drastically modify the properties of soils. Soils play a critical role in ecosystems, as they provide the environment where biological activity takes place, and their quality is of central importance in order to ensure sustainable food production.

Soil quality is defined as the capacity of a soil to function to sustain biological productivity while maintaining environmental quality (Doran and Parkin, 1994), and a decrease of quality is a serious environmental problem with direct consequences on the capacity of agricultural systems to provide food. For example, changes in soil quality have been reported to be highly correlated to crop yields (Mueller et al., 2013), and serious quality loss may reduce agricultural production by 60%, constraining the development of human populations (Eni, 2012). Land mismanagement and extractive farming may lead to a downward spiral of decreasing soil quality that could permanently affect agricultural productivity (Lal, 2015). If suitable measures are not applied to maintain soil quality, cultivation cost and the risk of crop failure increase, and land abandonment may follow (Grau and Aide, 2008; Joshi et al., 1996). Therefore, understanding how soil quality responds to land-use changes is important to improve land-use management, predict potential adverse effects on soil functioning and identify problem areas (Gregorich et al., 1994; Qi et al., 2009). It is also the basis for development of management practices aiming at the protection and restoration of soils in order to ensure long-term sustainable agricultural production, and meet the increasing food demand.

Soil properties and microbial processes are used as indicators of changes in soil quality. Particularly, soil microorganisms have been acknowledged as meaningful and early indicators of changes in soil functioning due to their close association with organic matter decomposition and nutrient cycling. Through their role in organic matter decomposition, soil microorganisms are responsible for regulating the provision of nutrients to plants. Furthermore, soil microorganisms contribute to the formation of soil aggregates, ultimately contributing to water and nutrient retention capacity and soil aeration. Evaluation of soil microbial properties and activities thus provide insights on changes in soil quality, and due to their sensitivity to environmental perturbations, they might be better indicators of alterations induced by land-use changes than soil physico-chemical variables alone (Nielsen and Winding, 2002).

Historically, potato production has been the main activity of the inhabitants of the Andean region of Bolivia (Hellin and Higman, 2005). Over the last fifty years the population of the country has more than tripled, leading to rapid land-use changes and agricultural intensification. Agricultural systems have experienced increasing pressure to meet the food demand of the population, especially due to high potato consumption and its central cultural role (Delgado & Delgado, 2014).

Decrease in soil quality for cultivation has been already acknowledged in the Andes, and farmers report decreasing potato yields, and the need to increase fertilizer applications from year to year to sustain productivity (Augstburger, 1983; Godoy, 1984). Degradation of soil quality affects the vulnerable populations that inhabit the region and the food security of the country, especially given that Bolivia reports one of the lowest average yields per hectare in South America (Scott, 2010). However, our understanding of the effects of the current land uses on ecosystems is scarce. Available information for the region focuses on soil physico-chemical characteristics and virtually no studies have evaluated soil quality and the potential consequences for agricultural production. Given the importance of these ecosystems in providing food to the country, evaluation of the effects of land-use changes and current agricultural practices on soil quality is essential to guide future management practices aiming at long-term sustainability.

Studies investigating the effects on land-use changes on soil quality often focus on one aspect of the physical, chemical or biological soil components only. Due to the close link between these components, changes of one will impact the others and lead to an overall decline in soil quality. Multivariate approaches for soil quality assessment in response to land-use changes can be more effective than single parameter evaluations (Bhardwaj et al., 2011), and are essential for a better understanding of soils. This thesis focused on the combined response of the soil physico-chemical and microbial characteristics to land-use changes. The study was conducted in an agricultural region located in the mountainous Central Andes of Bolivia. The region encompasses around 7000 potato farmers that provide potato for consumption and seeds to the country. We sampled soils of the main land uses that dominate the landscape: potato cultivated fields, fields under short fallow periods (2-6 years), and eucalyptus plantations. Further, we conducted a soil fertilization experiment to assess the effect of this practice on soil quality indicators.

Objectives and outline of the thesis

The main goal of this study was to assess the effects of fields cultivated with potato, short fallow fields (2-6 years old), *Eucalyptus globulus* L. plantations, and chicken manure fertilization on soil quality in agricultural ecosystems of the Central Andes of Bolivia. We evaluated soil physico-chemical properties, microbial processes and their interactions as indicators of changes in soil quality. It was hypothesized that leaving soils during 2-6 years under fallow would be too short to allow significant changes in soil quality compared to fields cultivated with potatoes, and that fertilization with chicken manure would improve soil quality for cultivation. It was also hypothesized that the introduction of the exotic tree *E. globulus* would reduce soil quality for cultivation compared to potato cultivated fields.

To fulfill the general objective this thesis was divided in 6 chapters:

In **Chapter 1**, a general introduction about the importance of soils and the maintenance of soil quality for sustainable food production is provided. The use of soil properties and processes as indicators of changes in soil quality is revised. A general description of the agricultural systems in the Central Andes of Bolivia and the land-use changes that occurred over the past decades are also provided.

In **Chapter 2**, we evaluated the differences in soil physico-chemical properties and microbial processes related to carbon and nitrogen mineralization of fields cultivated with potato, fallow fields and eucalyptus plantations. We also aimed at determining the soil physico-chemical indicators of changes in microbial processes. It was hypothesized that soil properties and microbial processes would be similar in potato crops and fallow fields while eucalyptus would promote carbon mineralization but reduce N mineralization rates. It was also hypothesized that labile water extractable fractions would be better indicators of soil processes related to carbon and nitrogen mineralization than other soil physico-chemical properties.

In **Chapter 3**, the effects of fields cultivated with potato, fallow fields and eucalyptus plantations on soil aggregate stability and size distribution were evaluated. It was hypothesized that eucalyptus plantations would lead to higher aggregate stability and abundance of bigger aggregate sizes, while potato and fallow land uses would lead to reduced stability and increased abundance of smaller aggregate sizes. We also aimed at investigating the soil constituents that explain variation of aggregate stability and size distribution, and whether microbial processes are associated to changes in aggregate size distribution. It was hypothesized that the microbial biomass carbon would be the soil constituent explaining most of the variation of aggregate stability and size distribution, and that higher microbial activity would be associated to bigger aggregate sizes.

In **Chapter 4**, we investigated the differences in abundance of ammonia-oxidizing archaea and bacteria in the three land uses studied (potato fields, fallow fields and eucalyptus plantations) and their implications for the nitrification process. We hypothesized that potato cultivated land use and fallow land use would lead to higher abundance and activity of

ammonia-oxidizing bacteria while eucalyptus plantations would lead to higher ammonia-oxidizing archaea abundance and activity.

In **Chapter 5**, we evaluated the effects of soil fertilization with chicken manure at different stages of decomposition and with varying turning frequencies on seed germination, plant growth and soil characteristics. It was hypothesized that chicken manure at early stages of decomposition would negatively affect seed germination and plant development but would increase the content of labile carbon and nitrogen of the soils compared to manure at late stages of decomposition. Further, we hypothesized that increased turning frequency would accelerate the composting process and reduce both the negative effects on seed germination and plant growth, and labile C and N contents in the soil.

In **Chapter 6**, a synthesis and general discussion of the results obtained in this thesis are presented. The changes in soil physico-chemical and microbial variables as soil quality indicators following the conversion of potato crops to fallow fields and to eucalyptus plantations are discussed. Further, the potential consequences of the changes in soil quality for agricultural production and long-term sustainability are discussed, as well as the perspectives for future research required for understanding agricultural ecosystems are provided. Finally, we discuss potential variables that can be used as indicators of changes in soil quality.

Chapter 1

General Introduction

1.1 Soil quality and ecosystem functioning

Soils are central components of ecosystems, as their physico-chemical and biological characteristics largely determine the ability of a soil to perform functions such as nutrient cycling, C-pool regulation, biodiversity and agricultural or forestry production (Greiner et al., 2017). These soil functions are the result of interactions between soil properties and processes. The combination of soil functions, in turn, contributes to the provision of ecosystem services, such as agricultural production or climate regulation, which provide direct benefits for humans (e.g. nutrition, health; Greiner et al., 2017). Therefore, soils are also an essential component for human development, and modifications of their physico-chemical or biological characteristics may have cascading effects on soil functions, ecosystem services, and human populations.

Soil quality is the capacity of a soil to perform functions within ecosystem and land-use boundaries, to sustain biological productivity, maintain environmental quality, and promote plant and animal health (Doran and Parkin, 1994). This concept was developed in response to the necessity to determine how soils responded to various management practices, and was envisioned as a tool for addressing the worldwide challenge of matching the food demand of the growing population in sustainable ways (Karlen et al., 2003). Due to the inherent differences among soils, there is no single definition of what characterizes "good" soil quality, and its definition also depends on the intended use of a given soil (Karlen et al., 2003). However, a soil with good quality for agricultural production may be defined as a soil that possess physical, chemical and biological characteristics that allow it to sustain long-term agricultural production (Reynolds et al., 2007). Improved soil quality for cultivation is generally associated with high soil organic matter content because the decomposition of organic compounds is the source of nutrients potentially available to plants. An active soil microbial community is also associated with good soil quality for cultivation because it determines the rate of organic matter decomposition and nutrient cycling. For example, the soil microbial biomass is a labile fraction, highly correlated with soil nutrients that are easily available to plants (Anderson & Domsch, 1980; Ghani et al., 2003; Joergensen, 2010; Stenberg, 1999). All soil organic matter should pass through the microbial biomass in order to be mineralized, therefore, microbial processes such as organic matter mineralization will determine the rate at which organic matter passes through this fraction and the availability of nutrients to plants. Furthermore, soil organic matter entering to the soil and the soil microorganisms that decompose it contribute to the formation aggregates, which in turn determine the porosity, water retention capacity and aeration of the soils.

A decline in soil quality implies soil degradation of the physical, chemical and biological soil components. Conceptually, a decrease in physical soil quality results in a change of structural characteristics, such as a reduction of soil aggregate stability and a higher abundance of smaller aggregate sizes, reduced pore density and increased risk of soil erosion (Lal, 2015; Oldeman, 1992). Physical degradation occurs when soil disrupting practices (e.g. tillage) break down soil aggregates. Altered soil structure may increase the risk of soil

erosion, soil compaction, and nutrient runoff (Tully et al., 2015). Decrease in chemical soil quality is commonly associated with intensified farming and poor management practices. It is characterized by nutrient depletion as the primary form of degradation, caused by unbalanced nutrient inputs and outputs (Lal, 2015; Tully et al., 2015). Loss of organic matter, acidification, decline of exchange capacity or cation imbalances are also signs of the degradation of chemical soil quality (Lal, 2015; Tully et al., 2015). Degradation of biological soil quality reflects changes in the abundance of soil microorganisms, microbial activity (e.g. respiration, mineralization, nitrification processes), as well as changes in the diversity of microbial groups linked to specific processes such as nitrification (Lal, 2015; Wurst et al., 2012). Similar to chemical degradation, changes in the biological component of soils can affect nutrient availability for plants and the complexity of food-webs, with consequences on ecosystem functioning. Due to the sensitivity of soil microorganisms to environmental changes, degradation of biological soil quality is caused by a wider range of factors than those causing soil physical and chemical soil quality degradation (e.g. tillage, clearing of vegetation, intensive cultivation, and climate perturbations).

Changes in soil quality are associated with changes in agricultural productivity. For example, several studies have reported a high correlation between soil quality and crop yields (de Paul Obade, 2019; Mueller et al., 2014; 2013). Due to the close link between the physical, chemical and biological soil components, degradation of one will impact the others, and lead to overall decline in soil quality. Feedbacks could also take place leading to an downward spiral of soil degradation, and compromising ecosystem sustainability (Lal, 2015). When the decrease in soil quality is severe, agricultural productivity has been documented to be reduced for as much as 60%, with considerable impact on wealth and economic development of human populations (Eni, 2012). Field abandonment commonly follows, and soils could be further degraded, especially in developing countries where the cost of soil recovery actions is restrictive (Grau and Aide, 2008; Harden, 1996).

Due to our dependency on ecosystems to produce food, maintenance of soil quality is essential to ensure the provision of ecosystem services and long-term sustainability (Karlen et al., 1997; Lal, 2015). Maintenance of soil quality requires basic information on the characteristics and interactions of the physical, chemical and biological soil components that contribute to soil quality. Soil quality evaluations are thus fundamental in order to guide soil management actions aiming at quality maintenance and long-term agricultural production.

1.2 Assessing of soil quality through the use of indicators

Since the recognition of the importance of assessing and monitoring soil quality, a plethora of assessment tools have been developed. Due to the specific characteristics of each soil, both inherent and dynamic soil properties and processes are considered to be essential for soil quality assessment (Karlen et al., 2003). However, a set of requirements for selecting quality indicators have been proposed. For example, Bünemann et al. (2018) stated that a meaningful indicator must be related to a specific soil function or ecosystem service, and that it should be sensitive enough to detect changes in soil functions in response to management practices or land-use changes. Moreover, assessment of soil quality indicators

should go beyond measuring a set of properties and processes, and should include their interactions and responses to changing conditions (e.g. such as land-use change; Bünemann et al., 2018). Information provided by the indicators would thus contribute to our understanding of the bi-directional interactions between the physical, chemical and biological soil components.

A common approach for soil quality assessments is the calculation of soil quality indexes, however, the compilation of results into a single value leads to loss of valuable information that could give insights on soil functioning (Bastida et al., 2008; Bünemann et al., 2018). Moreover, soil properties and processes should be weighted for their incorporation in a single index that could under- or overestimate their contribution to quality and lead to a biased index (Bastida et al., 2008; Bünemann et al., 2018). Given that soil quality is a soil property that emerges from the complex interactions between properties and processes, it is far from being an objective measure (Bastida et al., 2008). Soil quality assessments are thus more informative if presented as a multifaceted characteristics including physical, chemical and biological indicators (Lal, 2015).

1.2.1 Physico-chemical indicators of soil quality

Soil physico-chemical properties provide insights on the direct environment where soil microorganisms and plant roots develop, as well as on the potential constraints they could face (e.g. soil acidity/alkalinity, water and nutrient availability). They have been widely used for assessing changes in soil quality, although their sensitivity to changes in land use and management are highly variable. For example, total soil organic matter has been used as a central attribute of soil quality as it represents the primary source and sink for nutrients, as well as it contributes to soil porosity and water retention capacity (Doran and Parkin, 1994; Gregorich et al., 1994). Total soil organic matter, however, changes over long time periods and may not be sensitive enough to detect changes in soil quality in response to land use or soil management. In contrast, labile carbon fractions provide a measure of the pools that are more directly linked to microbial activity, such as organic matter decomposition, and they are thus subject to relatively higher turnover rates (Gregorich et al., 1994) and more sensitive to changes in land use or management. Therefore, due to the multi-faceted role of soil organic matter and labile fractions, their assessment is informative of soil functioning and quality (Curtin et al., 2021).

Similarly, soil pH, exchangeable cations and nutrient contents have also been used for assessing soil quality. Despite the buffering capacity of soils, soil pH may vary as a result of land-use change, or management practices such as fertilization (Zhao et al., 2020), and modifications may largely influence the solubility and availability of nutrients in the soil solution, ultimately affecting microbially related processes and plant nutrition (Robarge and Corey, 1979). Changes in exchangeable cations and nutrient contents are also indicative of changes in soil quality, as they are closely related to plant growth and biomass production. Similarly, evaluation of soil structure through the measurement of soil aggregate size distribution and stability contributes to soil quality assessments. Soil aggregates directly determine distribution, availability and stabilization of soil organic matter and nutrients, and

therefore play a central role in soil processes, such as microbial respiration, and functions, such as nutrient cycling (Tisdall and Oades, 1982).

1.2.2 Microbial indicators of soil quality

Soil microorganisms constitute a small fraction (between 1 to 3%) of total organic matter present in the soil (Kazuyuki and Yuhua, 2014). They are responsible for processes such as mineralization of organic compounds, and therefore play an essential role for soil functioning and the delivery of ecosystem services through their contribution to soil fertility and agricultural production. Given their sensitivity to environmental changes and to the pedological context, soil microorganisms have been used as indicators of changes in soil quality driven by land-use changes and intensification (Anderson, 2003; Krüger et al., 2017; Nannipieri et al., 2003). For example, soil microbial biomass increased after conversion from agriculture to grassland (Landgraf, 2001) or after afforestation (Deng et al., 2016). The microbial biomass provides information on the quantity of soil microorganisms present in soils, and is considered as "the eye of the needle through which all organic matter in the soil must pass" (Jenkinson, 1977). It will thus determine the potential of a soil to cycle nutrients, and their potential availability to plants. Furthermore, microbial activity measurements associated to the C cycle (e.g. respiration potential, the metabolic potential of soil microorganisms) complement information of the abundance of soil microorganisms, their metabolic status and potential contribution to C cycling and organic matter decomposition.

Nitrogen is commonly a limiting nutrient for plant growth and agricultural production, and at the same time, nitrogen forms such as nitrate, nitrite and nitrous oxide play an important roles in environmental pollution. Therefore, nitrogen transformations and the microorganisms involved in these processes have been used for assessing changes in soil quality (Schloter et al., 2003). For example, nitrogen mineralization is a microbial-driven process that mobilizes organically bound nitrogen (Ladd and Butler, 1972), and makes it available to plants and microorganisms. It is thus considered the limiting factor for the first step of the nitrification process, the oxidation of ammonia into nitrate (Barabasz et al., 2002). While N mineralization responds to changes in organic matter quality (its N content) and quantity entering to the soil (Luce et al., 2016; Scott & Binkley, 1997), nitrification responds to changes in the availability of ammonia. Consequently, both have been used to assess the effects of land-use changes or soil management practices, and give insights on changes in the N cycle. Moreover, changes in the abundance of the microorganisms involved in nitrification have also been used to assess the effects of land-use change and intensification on nitrogen cycling (Wurst et al., 2012) because this process is conducted by a restricted group of soil bacteria and archaea. Modification in N mineralization, nitrification and associated microorganisms, have been reported to impact biomass production (agricultural productivity) in diverse ecosystems. For example, Wessén et al. (2010) documented changes in the activity and diversity of ammonia-oxidizing microorganisms as a result of different fertilization practices, with consequent increases in N availability to plants and higher crop yields. Garcia-Montiel and Binkley (1998) reported changes in N availability following tree plantations on previously cultivated fields with effects on N cycling and ecosystem

functions. Evaluation of these nitrogen-related processes thus provide information on the nitrogen cycling function and potential side effects of its perturbations (ground water contamination, soil acidification, and on soil quality (Barabasz et al., 2002; Cakmak, 2002).

1.3 Land-use changes and intensification in the Central Andes of Bolivia

In Bolivia, agriculture has been the main activity of indigenous inhabitants of the Andean region, and their livelihood security has been based on the production of tubers, mainly potato (Hellin and Higman, 2005; Yarzábal et al., 2017). Today it is still the most important crop of Andean communities, as it occupies most of the available arable lands and labor force (Delgado & Delgado, 2014). Potato production is dominated by smallholders that provide food to local and national markets (Yarzábal et al., 2017). The traditional farming method was characterized by 3-4 cultivation cycles followed by long fallow periods of 10-50 years, and constituted a successful strategy that allowed high potato yields by restoring the fertility of cultivated fields (Pestalozzi, 2000; Sivila and Hervé, 1994; Zimmerer, 1993a). Nevertheless, the fast increase in population (the population more than tripled since 1950, Reed, 1998) led to increased food demand, with consequent land-use changes and intensification. Agricultural areas have thus been subjected to field subdivisions, increased cropping frequency and the dependency on external inputs for managing soil fertility (Kessler and Stroosnijder, 2006; Pestalozzi, 2000; Pijnenborg, 1998). Traditional agricultural practices were abandoned and currently fallow periods are no longer than 6 years.

Consequently, the use of external chemical and organic fertilizers was adopted as an alternative to manage soil fertility. During the decades of 1970 and 1990 the Bolivian state, through the Bolivian Institute of Agricultural Technology ("Instituto Boliviano de Tecnología Agropecuaria" IBTA), provided technical guidance to farmers for chemical fertilization, and introduced new potato varieties (Coca, 2012; Ortega and Rivas, 2004), contributing to agricultural intensification (Augstburger, 1983). However, due to the restrictive prize of chemical fertilizers, organic alternatives such as a chicken manure were preferred by farmers, and it has been widely adopted due to its availability, low cost and high nutrient content (Augstburger, 1983). Augstburger (1989) indicated that its residual effect allowed farmers to apply chicken manure once during several cultivation years, however, currently its use has increased and farmers apply manure once or more times in every cultivation cycle. Upon the definitive closure of the IBTA in 1997, support to agricultural production relied on cooperation projects and non-governmental institutions (Ortega and Rivas, 2004; Uzeda, 2005) unable to cover the need for technical guidance of the numerous agricultural communities of the Andean region. Soil fertilization practices now rely on communal decisions or farmers' personal judgement, which potentially lead to excessive fertilizer application (FAO, 1999) and consequent nitrate leaching, ground water contamination or soil acidification.

Furthermore, the exotic tree *Eucalyptus globulus* Labill was successfully introduced to Andean agricultural systems of the country at least 25 years ago through several governmental projects supported by international cooperation (Patiño, 2014). The main

objective of its introduction was the diversification of farmers' income, who historically relied on potato production (Patiño, 2014). Forestation programs were included as part of a national plan for economic and social development and were considered to be a sustainable alternative in rural communities (Fonabosque, 2021a; Ministerio de Medio Ambiente y Agua, 2010). Currently, forestation projects still contribute to the introduction of exotic tree plantations to agricultural systems (Fonabosque, 2021b; Villalobos, 2020).

Due to its fast growth rate and low water requirement, eucalyptus became highly valuable as energy source, especially for people living in peripheral areas of the communities (Flores, 2009; Morales and Patiño, 2008). Moreover, in comparison to other introduced tree species (*Pinus radiata* L.), eucalyptus can be thinned and the branches sold without killing the tree, which increases profits and attracted farmers' interest (Sandoval, 2008). Consequently, it was extensively planted on communal and private lands (Flores, 2009), replacing agricultural fields. Currently it is the main species used for plantations in the central Andean region of the country, particularly in the Cochabamba department, where the majority of the eucalyptus plantations are located (Sandoval, 2008).

Despite its initial high acceptability, over the years farmers have reported negative effects of eucalyptus plantations. For example, farmers perceive reduced potato productivity on lands previously planted with eucalyptus and lower economic income, as well as ecological impacts due to the scarce understory of eucalyptus plantations (compared to native forests or fallow fields; Flores, 2009). While extensive research was conducted to ensure successful tree plantations (Morales and Patiño, 2008; Patiño, 2014), available information on the effects of exotic trees on the soils of these Andean ecosystems is scarce.

These changes (shortened fallow periods, extensive use of organic fertilizers, and plantations of eucalyptus) might lead to changes in soil physico-chemical characteristics, microbial process and overall quality. So far, the efficiency of leaving soils in short fallow periods for fertility restoration has not been studied. Given that the time needed for nutrient restoration depends on the element considered (nitrogen can be restored within two years while cations may require more than 15 years, Styger & Fernandes, 2006; Szott & Palm, 1996), this practice may not contribute to soil fertility restoration and to potato production as expected, but may still preserve soil structure by reducing the risk of erosion once native vegetation covers the fields. Similarly, no information exists on the effects of fertilization practices on soil quality, particularly in view that chicken manure is added to the soil at different stages of decomposition (ranging from few days after its retrieval from the chicken farms up to 4-5 months of decomposition), and may or may not be subjected to proper compost management practices such as turning events. Also, the effects of eucalyptus on soil functioning in this area are not known, and while it may contribute to soil organic carbon buildup it could cause soil acidification, nutrient depletion (Leite et al., 2010), or induce allelopathic effects on plants and microorganisms (Cermelli et al., 2008; Zhang & Fu, 2010).

Currently, the information available for the agricultural highland ecosystems in Bolivia focuses on soil physico-chemical parameters only, while no studies have been conducted to determine the effects of these changes (shortened fallow periods, extensive use of organic

fertilizers, and plantations of eucalyptus) on overall soil quality. Lack of research oriented to understand these agricultural systems threatens their sustainability and food security (Yarzábal et al., 2017) as they supply food to the country. Moreover, the low average potato yields reported in Bolivia in comparison to neighboring countries with similar Andean ecosystems (Perú, Ecuador, Chile; Scott, 2010) reflects the disparities in cultivation strategies and highlights the potential to enhance agricultural production in the country. Comprehensive assessment of soil functioning under the current land uses (potato cultivated fields, fallow fields, and eucalyptus plantations), as well as the effects of fertilization practices in soil processes are thus essential to provide data helping in the decision making process of future soil management practices and land-use planning aiming at improving agricultural production, and at the same time, long-term sustainability.

1.3.1 Potential effects of agricultural intensification on soil quality

A major consequence of agricultural intensification is the disruption of soil functions and loss of ecosystem services (Grau and Aide, 2008). Due to the disruptive characteristic of conventional agriculture, changes in soil structure is one of the most noticeable effects that can lead to soil degradation through erosion, loss of organic matter and nutrient retention capacity (Tobiašová et al., 2016; Whalen and Chang, 2002). For example, agricultural land use has been largely reported to alter soil structure by changing aggregate size distribution and turnover rates through mechanical disruption (e.g. tillage, Chan et al., 2002; Wei et al., 2006). Such disrupting events have been associated to a flush in microbial activity that temporarily increases organic matter mineralization due to changes in carbon accessibility by microorganisms. In the long term, decreases of the soil microbial biomass and organic carbon pools are possible, and could further modify the microbial metabolic potential of the community as a consequence of changes in the quantity and quality of organic matter available to microorganisms (Kandeler et al., 1999).

Loss of soil organic matter in intensified agricultural systems has been associated with reduced nutrient retention capacity with direct effects on soil fertility and crop yields. Fertilization thus becomes an essential management practice aiming at replenishing lost nutrients and achieving high crop yields. While the use of organic fertilizers is commonly highly encouraged (Li et al., 2011) due to the positive effects on soil organic matter, nutrient content and soil structure (He et al., 2009; Peñaloza et al., 2019), inappropriate fertilization practices such as excessive fertilizer application or the use of raw manures may have potential environmental impacts. For example, organic fertilization may promote N mineralization and nitrification resulting in increased available N for plants (Hossain et al., 1995), but excessive fertilization in intensified agricultural systems may lead to soil acidification, ground water contamination with nitrate, or increased greenhouse gas emissions which could disrupt the overall soil functioning (Bolan et al., 1991; Kampschreur et al., 2010; Subbarao et al., 2012).

Leaving soils in fallow periods is also a common practice in agriculture that aims at the maintenance/restoration of soil quality (Barrios et al., 2005). Particularly in regions with low fertile soils, allowing fallow periods longer than 10 years have been reported to significantly

contribute to soil fertility and crop yields (Pestalozzi, 2000; Sivila and Hervé, 1994; Zimmerer, 1993a). Changes associated with fallowing include increases in soil microbial biomass, slower carbon turnover that leads to carbon stabilization, reduction of metabolic activity of the microbial community and lower N mineralization and nitrification rates (Pestalozzi, 2000). However, as pressure on agricultural soils increases to meet the food demand, fallow periods are shortened and the effectiveness of this practice to maintain/restore soil fertility and quality may diminish. Evaluation of the effects of agricultural intensification requires comprehensive assessment of soil properties and processes as indicators of changes resulting from management practices such as soil fallowing and fertilization.

1.3.2 Potential effects of eucalyptus on soil quality

Several studies have assessed the effects of eucalyptus plantations on soils, and both positive and negative effects have been reported across different environments. Eucalyptus plantations may contribute to soil organic matter accumulation and consequent increase in organic carbon pools. For example Bai and Blumfield (2015) and Paul et al. (2003) reported that eucalyptus plantations contribute to labile C pool restoration, subsequent increase in microbial biomass and carbon mineralization, overall contributing to a more active carbon cycling. Higher microbial activity in turn may promote the formation of soil aggregates and the improvement of soil structure (improved aeration, water infiltration and retention capacity). For example, significant reduction of the risk of soil erosion due to eucalyptus plantations compared with agricultural soils has been reported in mountainous areas (Jaleta et al., 2017). However, changes in other physico-chemical characteristics may counter the positive effect of organic matter accumulation. For example, accumulation or acid organic compounds may result in soil acidification and consequent changes in nutrients solubility and availability, as well as increased energy maintenance requirements of the microbial community. Moreover, disruption of the nitrogen cycle may also take place under acidic conditions or due to antibiotic and inhibitory compounds excreted by this tree that affect mineralization and nitrification processes (Bernhard-Reversat, 1988; Subbarao et al., 2009), reducing available nitrogen and ecosystem productivity (Barreto et al., 2012; López-poma et al., 2020). In contrast, other studies have suggest that eucalyptus produces short (Bai and Blumfield, 2015) and long-term fertilization effects by increasing in soil nitrogen pools and cations, such as potassium (Flores, 2009; Poore and Fries, 1987), but could also cause a decrease of cations such as Ca and Mg (Diaz et al., 2006).

The wide range of changes induced by eucalyptus on soils indicates that its overall effect on soil functioning and quality may vary between ecosystems, likely due to site-specific factors (e.g. soil type, climate). Understanding the effects of its introduction in Andean ecosystems on soil quality thus requires evaluation of physico-chemical and microbiological indicators, and cannot be inferred from previous studies or by studying isolated indicators of soil quality.

Chapter 2

Soil properties and microbial processes in response to land-use change in agricultural highlands of the Central Andes

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Abstract

Understanding changes in soil functions in response to land-use change is important for guiding agricultural practices towards sustainable soil management. We evaluated the differences in soil properties (soil organic matter, water extractable C and N, microbial biomass, pH_{KCL}, exchangeable cations) and microbial processes (respiration potential, net N mineralization, net nitrification, metabolic potential of soil bacteria), as well as the relative importance of soil properties in explaining changes in processes under three land uses (potato crops, fallow fields and eucalyptus plantations) in the agricultural highlands of the Central Andes. Soils under potato crops were characterized by the highest net N mineralization and net nitrification rates, extractable P, and the lowest microbial biomass P. Conversion to eucalyptus plantations led to an increase in soil organic matter, water extractable C, microbial biomass, and a decrease in extractable P and metabolic diversity of soil bacteria. Higher exchangeable Al indicated soil acidification under eucalyptus. Fallow practice did not lead to major changes in soil properties and microbial processes, indicating that fallow practices for up to 6 years were too short to substantially contribute to soil fertility restoration. The hot water extractable carbon showed the best relationship with soil processes (respiration potential, net N mineralization and net nitrification). Our results highlight the necessity of alternative management practices for maintaining soil fertility under potato crops, the drastic modification of soil properties and processes under eucalyptus plantations, and the potential of hot water extractable C as a proxy for monitoring land-use induced changes in soil functions related to C and N cycling.

Keywords: Bolivia, *Eucalyptus globulus*, Hot water-extractable carbon, Microbial activity, Microbial biomass, *Solanum tuberosum*.

2.1 Introduction

Soils are a central component of ecosystems, and the effects of land-use changes on soils have traditionally been assessed through their physico-chemical and biological properties, i.e. texture, chemistry, mass and abundance of organisms. These properties are determined by the basic constituents of the soils, mineral particles, organic matter, water and air. Recently, more emphasis has been placed on soil functioning, which refers to the ability of a soil to perform multiple soil functions, such as for example filtering of acids and pollutants, habitat provision, water cycle regulations, and nutrient cycling. Soil functions result from the interaction of soil properties and processes (Greiner et al., 2017), and they are related to ecosystem services and human benefits, as illustrated in the "Cascading framework" (Greiner et al., 2017; Haines-Young and Potschin, 2008).

Soil physico-chemical properties (e.g. microbial biomass, soil organic carbon, texture, pH, cation exchange capacity), microbial processes (e.g. carbon and nitrogen mineralization), and their interactions are used to assess soil functioning in relation to nutrient cycling (Brussaard, 2012; Wurst et al., 2012). While total soil organic matter and carbon are widely used indicators for assessing soil responses to land-use change, they are relatively insensitive to short-term changes (Muscolo et al., 2015, 2014). Furthermore, soil organic matter can be composed by 70-80% of a stable pool, resistant to microbial decomposition and might not be relevant for nutrient cycling (Haynes, 2005). In contrast, labile carbon fractions, such as microbial biomass, and water extractable C and N, act as substrates for microbial activity, and may be more relevant indicators for soil functions related to nutrient cycling (Haynes, 2005), but their use remains limited.

Alterations in physico-chemical soil characteristics due to land-use change will determine whether and to what extent microbial processes take place, which in turn will affect overall soil functions related to nutrient cycling. For example, agricultural land use may reduce soil organic matter, with a subsequent decrease in carbon mineralization rates (Beheshti et al., 2012; Paolini Gómez, 2018; Wang et al., 2013) , while increased nitrogen availability from fertilization may promote nitrogen transformations (Meinl et al., 2017). Conversion to fallow or afforestation, on the other hand, may lead to soil nutrient/carbon build-up and promote carbon and nitrogen mineralization rates (Cookson et al., 2007; Zhang et al., 2008). However, the response of soil processes to land use are context specific and dependent on the relative importance of soil physico-chemical properties in driving processes. The assessment of the relationships between soil properties and processes under different land uses is thus essential to achieve sustainability, as stated within the World Soil Charter (FAO and ITPS, 2015).

In the high mountainous areas of the mesothermic valleys of Bolivia (eastern branch of the Andes range), agriculture is the main professional activity for about 71% of the rural population (INE, 2015), with potato (*Solanum tuberosum* L.) as the main crop. During the last decades, these agricultural systems have been subjected to land-use change and intensification due to population increase and higher global food demand (Kessler and Stroosnijder, 2006). Changes include field subdivisions, increased cropping frequency and

the dependency on external fertilizer inputs for managing soil fertility (Kessler and Stroosnijder, 2006; Pestalozzi, 2000; Pijnenborg, 1998). Furthermore, *Eucalyptus globulus* L. varieties adapted to the Andean climate were introduced to diversify the farmers' income (Patiño, 2014), reducing the land available for annual crop cultivation, and contributing to the pressure on remaining arable soils. Concomitantly, the traditional farming method characterized by 3-4 cultivation cycles followed by long fallow periods of 10-20 years (Pestalozzi, 2000; Sivila and Hervé, 1994; Zimmerer, 1993a) was abandoned, and fallow periods were shortened to less than 6 years. Negative consequences of such agricultural intensification have been reported, for instance the increased risk for soil erosion, reduced soil fertility and crop yield (Kessler and Stroosnijder, 2006). As the current fallow periods have been shortened, they may not be sufficient for soil fertility restoration. Indeed, the time needed for nutrient restoration varies across elements; nitrogen may be restored within two years while cations may require more than 15 years (Styger and Fernandes, 2006; Szott and Palm, 1996). Moreover, while eucalyptus may contribute to soil organic carbon buildup and decreased soil erosion (Barros Soares et al., 2019; Jaleta et al., 2017), it may also cause soil acidification, nutrient depletion (Leite et al., 2010), or induce allelopathic effects on plants and microorganisms (Cermelli et al., 2008; Zhang & Fu, 2010). So far, the information available for the agricultural highland ecosystems in Bolivia focuses on soil physico-chemical parameters only and information on the effect of land uses on soil properties and microbial processes is missing. Given the importance of these ecosystems in supporting food security for the country, an assessment of the current soil properties and processes under the main land uses is needed to provide data helping in the decision making process of future soil management practices.

The aims of this study were to assess differences in soil functions related to carbon and nitrogen cycling following the conversion of potato crops to fallow fields and to eucalyptus plantations in agricultural highlands of the Central Andes. To assess soil function, we evaluated the association between soil properties and microbial processes related to carbon and nitrogen mineralization. We hypothesized that soil properties and microbial processes would be similar in potato crops and fallow fields because the fallow periods are now too short to induce significant soil improvement, and that eucalyptus might lead to organic matter build up, soil acidification, and reduced N mineralization. Also, we hypothesized that labile water extractable fractions might be better indicators of soil microbial processes than total soil organic matter content.

2.2 Materials and methods

2.2.1 Study site and soil sampling

The study was conducted in the fields of the Chullchunqani Community (17°32'30"-17°33'30" S, 065°20'08"-065°21'36" W, Figure 2.1), which belongs to a traditional agricultural region that encompasses ca. 7000 Quechua-speaking potato farmers. The community (ca. 50 families) has an organizational structure for strategic decision-making concerning potato production as part of economic risk minimizing strategies (Ellis-Jones

and Mason, 1999). The study site is located in the Puna biogeographic province in the Eastern branch of the Andes range, at an altitude of 3100-3400 m a.s.l. (Navarro and Maldonado, 2002), with soils classified as Cambisols (Ministerio de Medio Ambiente y Agua, 2014). The region is characterized by a summer rainy season (November-March) and a winter dry season (April-October) (Navarro and Maldonado, 2002; Pestalozzi, 2000), with a mean annual rainfall of 500.7 mm, and a mean annual temperature of 17.9 °C (SENAMHI, 2016). During the winter season average precipitation is low (16.0 mm), average temperature is 16.0 °C, and frost events can take place (SENAMHI, 2016).

These climatic conditions determine the rotation cycle: potato (*Solanum tuberosum* L.) is grown mainly during the rainy season (Coûteaux et al., 2008) and secondary crops (*Vicia faba* L., faba beans; *Hordeum vulgare* L., barley) during the dry season (a rotation calendar is included in S1 of supplementary material; Condori et al., 1997). Potato fields are tilled (ca. 20 cm depth) for soil preparation, and industrial N, P, K fertilizer inputs as well as chicken manure (average N, P, and K content: 3.5, 2, and 2.6%, respectively) are added at planting. Additional fertilization and irrigation is applied in varying amounts according to farmers' personal judgement, and harvesting is conducted manually. Before conversion to fallow or to eucalyptus plantations, plots were managed as cultivated fields. When converted to fallow the remaining aboveground biomass is incorporated by tillage and the fields are then left unmanaged. Fields converted to eucalyptus plantations are not managed after seedling plantation. The conversion of crops to eucalyptus plantations was not restricted to low fertility fields.

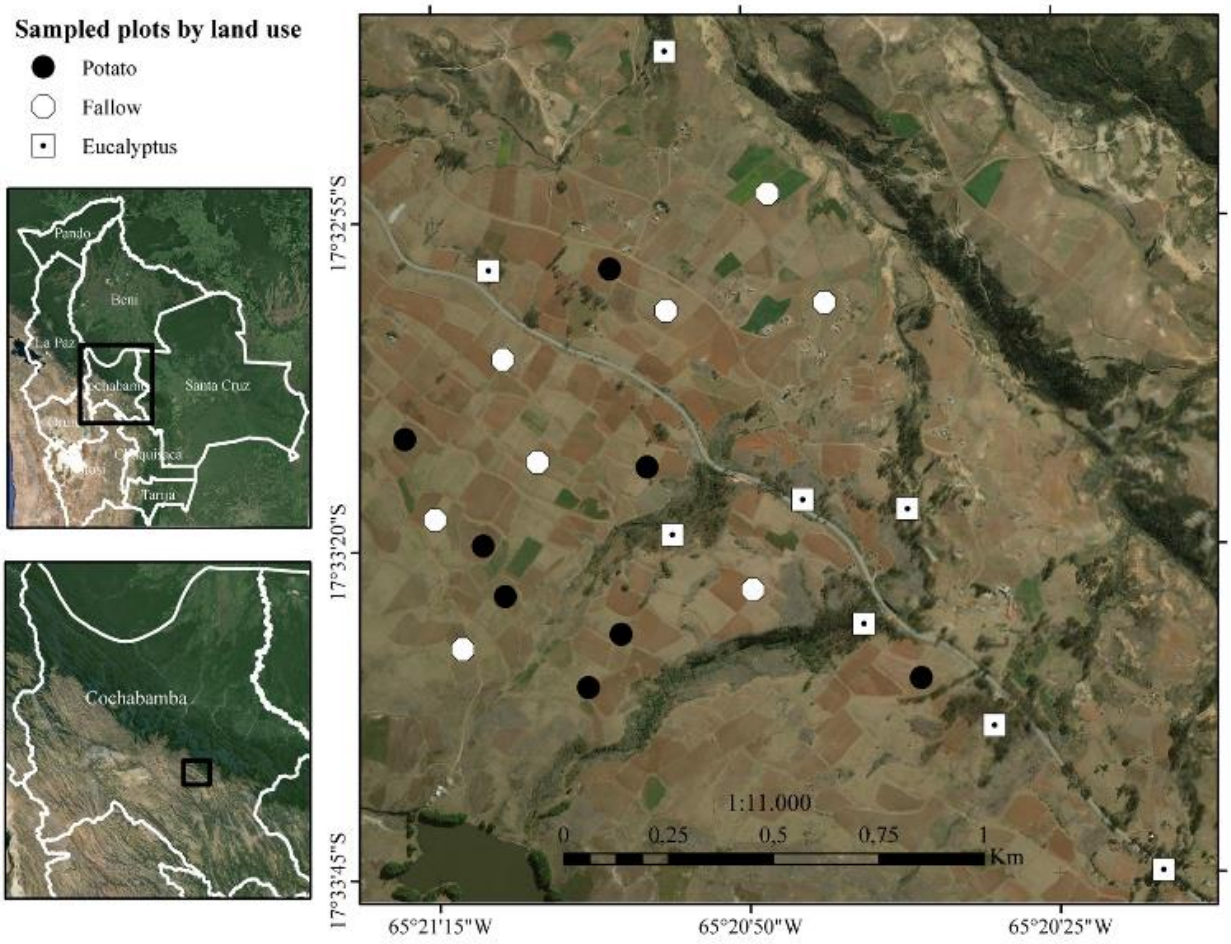

Figure 2.1 Satellite image of the study area. Location of the potato, fallow and eucalyptus plots in the Chullchunqani Community, which belongs to the agricultural region of Pocona Municipality (Cochabamba-Bolivia).

Potato, fallow and eucalyptus fields are interspersed within the landscape in areas of ca. 0.5-1 ha defined here as "plots". Twenty four plots (8 plots of each land use type) were selected within an area of ca. 4 km^2, based on the following criteria: potato plots, in which potato had been grown during the last rainy season (2016-2017); fallow plots (2-6 years old) in which the spontaneously grown vegetation (grassland of semiarid high Andes) fully covered the soil; and forested plots with *E. globulus* (5-25 years old). Soils from potato, fallow and eucalyptus land uses were sampled at the end of the rainy season (February 2017), 2-3 weeks after harvesting in the potato plots. Each plot was divided into 10 m quadrants, three were randomly selected and designated as sampling points. At each sampling point, one composite soil sample was taken with a shovel (one central sample and four individual samples taken two meters around; 20 cm depth), leading to a total of 72 soil samples. In order to compare similar soil layers, the thin organic layer (<0.5 cm) of the eucalyptus plots was discarded. Samples were homogenized, sieved (2 mm mesh) and stored at 4 °C.

2.2.2 Soil properties

General soil characteristics (soil texture; water holding capacity, WHC) were measured on one of the three samples taken in each plot (n=8 for each land use). Soil texture was determined with the Bouyoucos hydrometer method for particle size determination (Bouyoucos, 1927; McKean, 1993). WHC was estimated by the Shaw's method (Jenkinson and Powlson, 1976).

All other analyses were performed on all 72 samples (3 samples per plot). Gravimetric moisture content was determined by weight difference of 5 g of fresh soil samples dried at 105 °C for 4 h (Allen, 1989). Soil pH_{KCl} was determined with a pH-meter (HI2550 HANNA instruments) in 15 g fresh soil on a 1:2 (w/v) soil and KCl 1 M solution ratio (Allen, 1989). Soil organic matter (SOM) was determined on oven-dried samples by loss-on-ignition at 450 °C overnight, and total organic carbon was calculated as 58% of SOM (Allen, 1989). Water extractable carbon and nitrogen were determined using a sequential extraction, first at room temperature, followed by a second extraction at 80°C. Water soluble carbon and nitrogen were extracted from 10 g fresh soil, with 60 ml distilled water at room temperature (Ghani et al., 2003). The soil solutions were agitated (120 rpm, 30 min) centrifuged (4000 rpm, 10 min), and the supernatants were filtered and stored for chemical analyses. The remaining soil was re-suspended in 60 ml distilled water, and placed in an oven at 80 °C for 16 hours to determine the hot water extractable carbon and nitrogen (Ghani et al., 2003). Solutions were agitated and centrifuged as described above. The water soluble organic carbon (WSC) and hot water extractable organic carbon (HWC) were determined by measuring the total organic carbon in of the extracts with a Total Carbon analyzer (UV-persulfate method, Lab Toc, Pollution and Process Monitoring, UK). The NH_4^+-N, NO_3^--N and total nitrogen in the extracts were analyzed colorimetrically using a continuous flow analyzer equipped with an UV digestor (AutoAnalyzer 3, BranLuebbe, Germany) for water soluble total nitrogen (WSN_{tot}), and hot water extractable total nitrogen (HWN_{tot}) determination. Water soluble organic nitrogen (WSN_{org}) was calculated as the difference between the total and mineral N (NH_4^+-N, NO_3^--N). As mineral nitrogen is mostly removed in the first extraction step, and as NH_4^+ in hot water extracts may result from the hydrolysis of organic N (Gregorich et al., 2003), we assumed that all HWN_{tot} derived from organic N. Therefore, we used WSN_{org} and HWN_{tot} in our data analyses.

Exchangeable base cations (Ca^{2+}, K^+, Mg^{2+}, Na^+) and Al^{3+}, Fe^{3+}, Mn^{2+} were determined with the barium chloride extraction method (Hendershot and Duquette, 1986). Four grams fresh soil were shaken with 40 ml 0.1 M $BaCl_2$ (30 min at 180 rpm), filtered and the extracts were analyzed with an inductively coupled plasma atomic emission spectrometer (VARIAN Vista). Exchangeable base cations (EBC) was computed as the sum of Ca^{2+}, K^+, Mg^{2+}, Na^+. Extractable phosphorous was estimated with the $NaHCO_3$ extraction (Brookes et al., 1982; Horta and Torrent, 2007) of the non-fumigated samples for the determination of soil microbial biomass P (see below).

Soil microbial biomass carbon (MBC), nitrogen (MBN) and phosphorus (MBP) were determined with the chloroform fumigation extraction method (Brookes et al., 1982; Vance

et al., 1987). Fumigation of soil subsamples was carried out for 3 days in a vacuum desiccator with alcohol-free chloroform. For MBC and MBN, 10 g soil of fumigated and non-fumigated samples were extracted with 50 ml 0.5 M K_2SO_4 (1 h shaking at 180 rpm and filtration through Whatman filter #42). Organic carbon in the extracts was measured with a Total Organic Carbon analyzer (Lab Toc, Pollution and Process Monitoring, UK), and total nitrogen was analyzed colorimetrically using a continuous flow analyzer equipped with an UV digestor (AutoAnalyzer 3, BranLuebbe, Germany). For MBP, 8 g soil of fumigated and non-fumigated samples were extracted with 40 ml of 0.5 M $NaHCO_3$ (45 min shaking at 120 rpm and filtration through Whatman filter #42). Extractable phosphorus in the solutions was analyzed with an inductively coupled plasma atomic emission spectrometer (VARIAN Vista). MBC, MBN and MBP were calculated as the difference of fumigated and non-fumigated samples with a conversion factor of 0.45 for MBC (Joergensen, 1996), 0.54 for MBN (Brookes et al., 1985), and 0.4 for MBP (Brookes et al., 1982). The MBC:MBN, MBC:MBP and MBN:MBP ratios were calculated on molar basis.

2.2.3 Soil microbial processes

The soil respiration potential was measured as CO_2-C accumulation in the headspace (125 ml) of an amber bottle (Supelco, USA) from 20 g fresh soil, at 20 °C in the dark after an overnight pre-incubation (Robertson et al., 1999). Gas samples (4 ml) were taken at 0, 120, 150, 180, and 210 min with an airtight syringe (Hamilton Model 1005) and analyzed with an infrared absorption gas analyzer (EGM-4 PPsystem, UK). The respiration potential was estimated by linear regression of CO_2-C against time. The microbial quotient (qmic, an indicator of soil C availability to microorganisms; Anderson, 2003) was calculated by dividing the microbial biomass carbon by the total organic carbon content estimated as 58% of SOM (Allen, 1989). The metabolic quotient (qCO_2, an indicator of the quantity of respired CO_2-C per unit of soil microbial biomass, reflecting maintenance energy requirement) was calculated by dividing the respiration potential by MBC (Anderson, 2003; Anderson & Domsch, 1989, 1990).

Nitrogen transformations driven by soil microorganisms were assessed with an aerobic laboratory incubation essay (Hart et al., 1994a). Nitrogen was extracted from 15 g subsamples of fresh soil with 1 M KCl solution (1:5; w:v), after 1 h agitation at 180 rpm and centrifugation at 4000 rpm. In parallel, 15 g subsamples of fresh soil, adjusted to 60% water holding capacity, were incubated in the dark at 20 °C for 28 days. The water loss was monitored gravimetrically and compensated by adding distilled water as necessary. At the end of the incubation period, nitrogen was extracted as described above. Extracts were analyzed colorimetrically for NH_4^+-N, NO_3^--N using a continuous flow analyzer equipped with an UV digestor (AutoAnalyser3, BranLuebbe, Germany). Net nitrogen mineralization (Nmin) and net nitrification were calculated as the net increase in mineral nitrogen (NH_4^+-N and NO_3^--N) and nitrate (NO_3^--N) respectively, over the incubation period. The relative nitrification was calculated as the percentage of NO_3^--N produced relative to the total mineral N produced.

The metabolic potential of soil bacteria (a functional diversity, indicating the potential of soil bacteria to degrade different carbon substrates; Garland & Mills, 1991; Preston-Mafham et al., 2002) was determined with BIOLOG Ecoplates (BIOLOG^{TM}, California) containing one control well and 31 wells with different carbon substrates and tetrazolium dye (in triplicate), which indicates bacterial respiration by changing from colorless to purple. Fungi do not respond to the EcoPlate assay because they cannot reduce the tetrazolium dye included in the substrate (Preston-Mafham et al., 2002; Zak et al., 1994). One g soil was extracted with 9 ml 0.1% sodium cholate (Insam and Goberna, 2004) and diluted to 10^2, 10^3, and 10^4 with 0.85% NaCl to determine the number of colony forming units (CFU) on R2A agar (Insam and Goberna, 2004). Wells were inoculated with 100 µl of the dilution containing 1000-2000 CFU and incubated for 72 h at 20 °C. Absorbance values at 590 nm from each well were read with SynergyMx (BIOTEK instruments - USA). Blank values were subtracted from the readings of each sample, and a threshold for positive tests was defined as 0.25 absorbance units to eliminate weak positives (Garland, 1997, 1996). The overall rate of substrate utilization was estimated as the average well color development (AWCD), calculated by the mean value of single-point absorbance readings per sample (Insam and Goberna, 2004).

2.2.4 Statistical analyses

Differences of soil textural components sand, silt and clay, and WHC between land uses were assessed with simple ANOVA and Tukey tests (one sample per plot, ANOVA statistics are presented in S2 of supplementary material).

The differences between land uses of all other variables (3 samples per plot) were evaluated with nested ANOVA using linear mixed-effects models (LMM; Mangiafico, 2015). Models were constructed including land use as fixed effect and plot as random effect, which accounted for the non-independence of the three samples taken in each plot, and for local differences between plots (i.e. time under the specific land-use type). Logarithmic (Al^{3+}, Mg^{2+}, Mn^{2+}, HWC, respiration potential) and square root data transformations (Na^{+}, Fe^{3+}) were applied to fulfill ANOVA assumptions. Marginal R^2 ($R^2_{LMM(m)}$, variance explained by fixed effects) and conditional R^2 ($R^2_{LMM(c)}$, variance explained by both fixed and random effects) were calculated according to (Nakagawa and Schielzeth, 2013). The proportional variance associated with the random effect component ($R^2_{LMM(r)}$) could then be computed as $R^2_{LMM(r)} = R^2_{LMM(c)} - R^2_{LMM(m)}$, and the unexplained error was estimated as $\varepsilon = 1.0 - R^2_{LMM(c)}$ (detailed statistics are presented in S3 of supplementary material). Visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality. Post-hoc comparisons were conducted with Tukey test for mixed effects models (Faria et al., 2018; Mangiafico, 2015).

The overall multivariate discrimination between land uses was assessed through standardized principal component analyses (PCA) on all the variables measured (exept WSN_{tot}).

To determine the relative importance of the soil properties in explaining soil microbial processes, we constructed LMM using microbial processes (potential respiration, net N mineralization and net nitrification, and AWCD) as dependent variables. Soil properties (SOM, pH_{KCl} and EBC) were used as fixed effects. As the different carbon fractions were highly correlated (supporting information S4), separate models were constructed using pH_{KCl} and EBC, with either SOM, HWC, or MBC. For net N mineralization and net nitrification one model including HWN_{tot} instead of HWC was also fitted. HWC:HWN_{tot} ratio was not included as explanatory variables because the range of values was limited, all values were below the critical threshold of 20 and as their individual correlations with soil processes were not significant. To account for the dependence of the three samples taken within each plot, and the dependence of samples within each land use, both levels were included as random effects. Models were fitted using REML estimation and the marginal r-square $R^2_{LMM(m)}$ (variance explained by fixed effects), semi-partial R^2_i (variance explained by an individual predictor while adjusting for the other predictors in the model) were calculated according to Jaeger et al. (2016).

Statistical analyses were conducted with the R software 3.6.1 (R Core Team, 2018) using the packages "car" (Fox and Weisger, 2011), "nlme" (Pinheiro et al., 2018), "mgcv" (Wood, 2017), "TukeyC" (Faria et al., 2018), "multcomp" (Hothorn et al., 2017), "multcompView" (Graves et al., 2015), "MuMIn" (Barton, 2018), "r2glmm" (Jaeger et al., 2016), and "factoextra" (Kassambara and Mundt, 2019).

2.3 Results

2.3.1 Soil properties

For the general characterization of soils (Table 2.1), WHC and texture no significant differences were found between land uses.

Table 2.1 Mean values ± standard deviation of sand, silt, and clay fractions, water holding capacity (WHC). Different letters indicate significant differences between land uses (p-value <0.05, n=8, ANOVA and Tukey).

	Potato	Fallow	Eucalyptus
Sand (%)	21.80±4.84 a	22.85±4.29 a	34.08±9.64 a
Silt (%)	29.51±3.87 a	31.57±8.75 a	29.53±5.02 a
Clay (%)	48.69±5.88 a	45.57±11.30 a	36.38±6.26 a
WHC (%)	51.20±4.02 a	53.20±2.60 a	48.79±7.73 a

The SOM content, measured by loss-on-ignition, was significantly higher under eucalyptus plantations compared to potato and fallow plots, and ranged from 55.5 to 144.4 g kg^{-1} (Table 2.2). The soil exchangeable cations were dominated by Ca^{2+} (61%), Mg^{2+} (26%) and K^+ (11%) with significantly lower values of Ca^{2+} and K^+ under eucalyptus compared to potato

plots. Na^+ accounted for less than 1% of exchangeable cations and did not show differences between land uses. Exchangeable Al^{3+} was significantly higher under eucalyptus, accounting for 17% of exchangeable cations compared to potato and fallow plots where Al^{3+} accounted for 3 to 4%. The remaining cations Fe^{2+}, Mn^{2+} had significantly higher values under eucalyptus. The $NaHCO_3$ extractable P and the sum of exchangeable base cations (EBC) were significantly lower under eucalyptus plantations compared to potato and fallow plots. Soil pH_{KCl} and exchangeable Mg^{2+} were not significantly different between land uses (Table 2.2).

HWC was significantly higher under eucalyptus plantations compared to potato and fallow plots (Table 2.2). On average, the contribution of HWC to the total water extractable C (HWC+WSC) was 95, 93 and 93% under eucalyptus, fallow and potato plots respectively. HWC represented 2.13, 1.66, and 1.54% of total organic carbon (calculated as 58% of SOM; Allen, 1989) under eucalyptus, fallow and potato plots respectively. The HWN_{tot} fraction in eucalyptus and fallow plots accounted for 84 and 72% of total extractable N (WSN_{tot} + HWN_{tot}) with significantly lower values in potato plots where it accounted for 54% of total extractable N. On average, the WSN_{tot} was composed by 13, 3, and 2% of NH_4^+-N, and 55, 79, 90% of NO_3^--N under eucalyptus, fallow, and potato plots, respectively. WSN_{org} accounted for 6.7, 17.3 and 21.7% of WSN_{tot} in potato, fallow and eucalyptus plots. $HWC:HWN_{tot}$ and $WSC:WSN_{org}$ were significantly higher under eucalyptus plantations compared to potato and fallow soils. We did not find significant difference between land uses for WSC, WSN_{tot}, WSN_{org}, and HWN_{tot} (Table 2.2).

MBC ranged from 156.0 to 948.0 mg C kg^{-1}, and MBN ranged from 28.3 to 180.7 mg N kg^{-1}; both were significantly higher in eucalyptus plots. MBP values were below the detection limit for some soil samples in potato plots, and significantly higher values were recorded in eucalyptus plantations, with a maximum of 31.7 mg P kg^{-1}. The MBC:MBN molar ratio ranged from 3.0 to 13.5 with significantly lower values under eucalyptus compared to potato soils. The MBC:MBP and MBN:MBP had considerably higher variation and did not show significant differences between land uses.

2.3.2 Soil microbial processes

Soil respiration potential ranged between 0.15 to 1.07 µg CO_2-C h^{-1} g^{-1}, with the highest mean values under eucalyptus plots compared to fallow and potato plots (Figure 2.2). The microbial quotient qmic ranged from 3.84 up to 12.37 mg MBC g^{-1} soil C with higher values in eucalyptus plantations compared to potato and fallow plots, while the metabolic quotient qCO_2, ranged from 0.51 to 1.57 µg CO_2-C h^{-1} mg^{-1} C with significantly higher values in eucalyptus and fallow plots. Net N mineralization and net nitrification were significantly lower under eucalyptus and fallow compared to potato plots. Net NO_3^--N production accounted for 96% of the total net mineral nitrogen produced in potato and fallow soils while it accounted for 59% under eucalyptus. The metabolic potential of soil bacteria (AWCD) had significantly lower values in eucalyptus plantations compared to potato and fallow soils.

Table 2.2 Mean values ± standard deviation of soil properties. Values were calculated using average values of the three samples per plot (n=8). Different letters indicate significant differences between land uses (p-value <0.05, nested ANOVA using linear mixed-effects models and Tukey tests). Soil organic matter (SOM), exchangeable base cations (EBC), $NaHCO_3$ extractable phosphorus (extractable P) hot water extractable carbon (HWC) and nitrogen (HWN_{tot}), water soluble carbon (WSC), water soluble total nitrogen (WSN_{tot}) and organic nitrogen (WSN_{org}), soil microbial biomass carbon (MBC), nitrogen (MBN) and phosphorus (MBP).

	Potato	Fallow	Eucalyptus
Soil chemistry			
pH_{KCl}	4.34±0.28 a	4.30±0.30 a	3.96±0.23 a
SOM (g kg^{-1})	86.98±13.85 b	78.22±12.90 b	106.87±25.27 a
EBC ($cmol_c$ kg^{-1})	8.22±1.00 a	7.17±2.00 a	5.85±2.60 b
Ca^{2+} ($cmol_c$ kg^{-1})	5.23±0.28 a	4.69±0.53 ab	3.55±0.75 b
K^+ ($cmol_c$ kg^{-1})	0.88±0.13 a	0.87±0.12 a	0.52±0.08 b
Mg^{2+} ($cmol_c$ kg^{-1})	2.05±0.07 a	1.60±0.16 a	1.70±0.19 a
Na^+ ($cmol_c$ kg^{-1})	0.07±0.01 a	0.02±0.01 b	0.08±0.01 a
Fe^{3+} ($cmol_c$ kg^{-1})	$1.3x10^{-03}$±$7.71x10^{-04}$ a	$1.8x10^{-03}$±$8.9x10^{-04}$ ab	$2.9x10^{-03}$±$1.7x10^{-03}$ b
Mn^{2+} ($cmol_c$ kg^{-1})	$1.04x10^{-02}$±$5.3x10^{-03}$ a	$1.1x10^{-02}$±$7.2x10^{-03}$ a	$2.4x10^{-02}$±$1.5x10^{-02}$ b
Al^{3+} ($cmol_c$ kg^{-1})	0.23±0.05 b	0.28±0.03 b	1.19±0.37 a
Extractable P (mg kg^{-1})	63.47±18.66 a	38.69±8.42 b	18.59±8.02 c
Water-extractable carbon and nitrogen			
HWC (mg C kg^{-1})	774.00±129.49 b	750.80±186.16 b	1367.00±557.44 a
HWN_{tot} (mg N kg^{-1})	67.63±13.00 a	65.44±13.14 a	84.20±21.30 a
HWC:HWN_{tot} ratio	11.58±1.00 b	11.38±1.03 b	15.68±2.42 a
WSC (mg C kg^{-1})	56.67±16.58 a	55.23±17.76 a	69.41±19.59 a
WSN_{tot} (mg N kg^{-1})	55.81±16.74 a	25.36±7.50 b	19.116±12.05 b
WSN_{org} (mg N kg^{-1})	3.50±1.89 a	4.39±0.84 a	4.15±1.95 a
WSC:WSN_{org} ratio	15.29±5.13 ab	12.50±2.83 b	18.88±6.64 a
Soil microbial biomass			
MBC (mg C kg^{-1})	350.22±42.20 b	333.10±67.76 b	599.97±200.31 a
MBN (mg N kg^{-1})	53.56±13.17 b	63.37±14.06 b	118.20±34.57 a
MBP (mg P kg^{-1})	5.25±1.83 c	12.38±8.75 b	18.71±2.31 a
MBC:MBN	8.67±2.90 a	6.53±2.34 ab	5.85±0.56 b
MBC:MBP	238.16±245.82 a	135.29±106.55 a	104.53±74.71 a
MBN:MBP	30.54±28.26 a	19.37±13.17 a	17.56±11.58 a
MBC:MBN:MBP	238:31:1	135:19:1	105:18:1

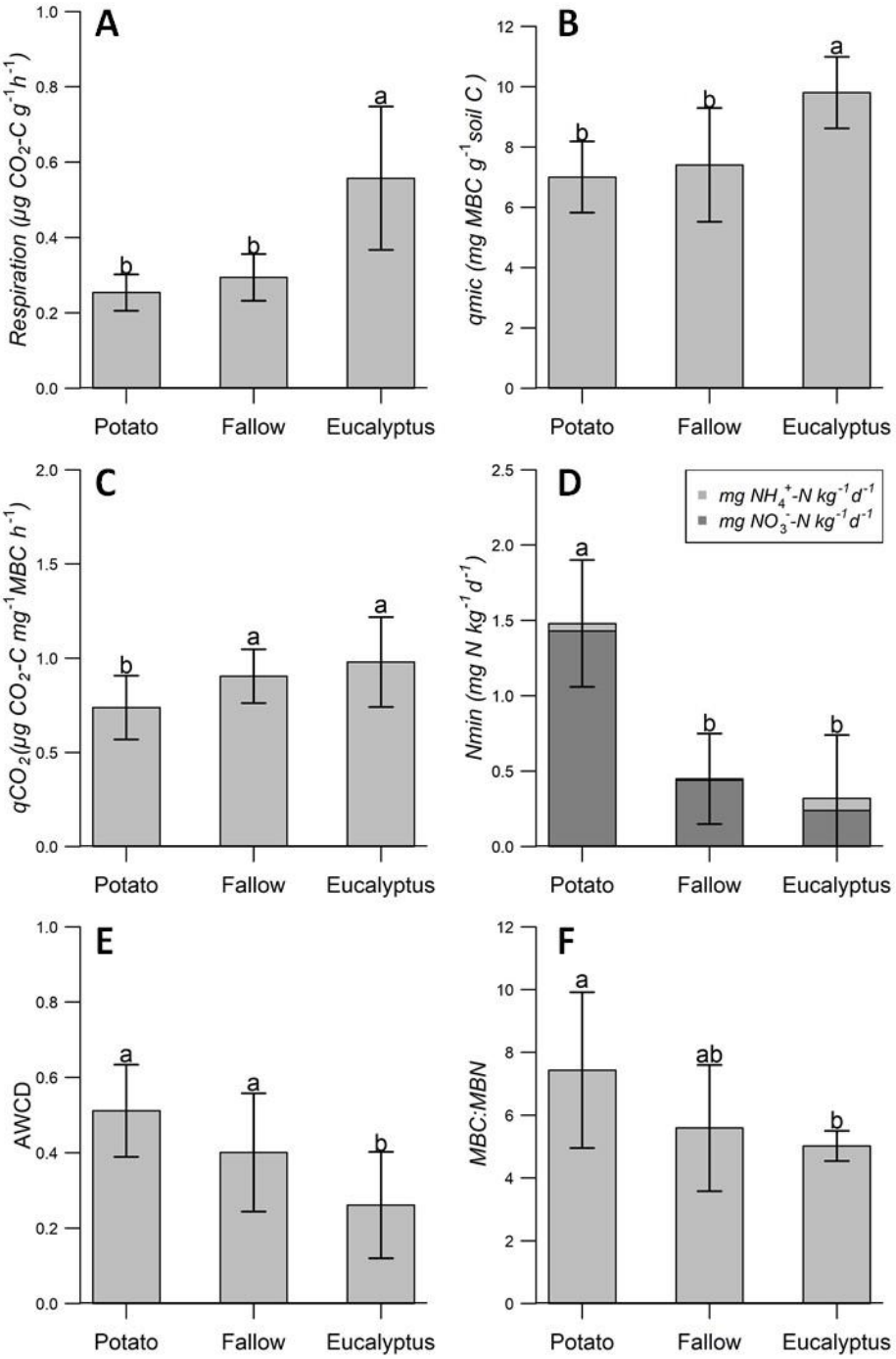

Figure 2.2 Soil processes and related parameters under potato, fallow and eucalyptus land uses. A: respiration potential, B: microbial quotient (qmic), C: metabolic quotient (qCO2), D: net N mineralization and net nitrification, E: average well color development (AWCD), and F: microbial carbon-to-nitrogen ratio. Mean values and standard deviations were calculated using average values of the three samples per plot (n=8). Different letters indicate significant statistical differences (p-value <0.05, nested ANOVA using linear mixed-effects models and Tukey tests).

2.3.3 Relationships of soil properties and soil processes

The PCA showed multivariate discrimination of eucalyptus from potato land use, while fallow land use was intermediate (Figure 2.3). The variable loadings indicated that eucalyptus land use was associated with labile C and N fractions, respiration potential, and exchangeable Al, Fe and Mn. Potato land use was associated with high net N transformation rates, WSN_{tot}, exchangeable base cations, extractable P and pH_{KCl}.

The results of statistical modeling to examine the relative importance of soil properties in explaining soil processes are presented in Table 2.3. pH_{KCl} and EBC were only statistically significant for AWCD. None of the carbon fractions (SOM, HWC and MBC) was a significant predictor of AWCD. Model fit was around $R^2_{LMM(m)}$=0.4 for all AWCD models, with a relatively higher contribution of pH_{KCl} compared to EBC for two of the models. Respiration potential, net nitrogen mineralization and net nitrification showed best model fit with HWC. HWC was the best predictor for respiration potential ($R^2_{LMM(m)}$=0.81), followed by MBC ($R^2_{LMM(m)}$=0.66) and SOM ($R^2_{LMM(m)}$= 0.37). For net N mineralization and net nitrification, HWC and HWN_{tot} were the best predictors (HWC: $R^2_{LMM(m)}$=0.49 and 0.50; HWN_{tot}: $R^2_{LMM(m)}$=0.47 and 0.54, respectively), followed by SOM ($R^2_{LMM(m)}$=0.31 and 0.33), and MBC ($R^2_{LMM(m)}$=0.17 and 0.19).

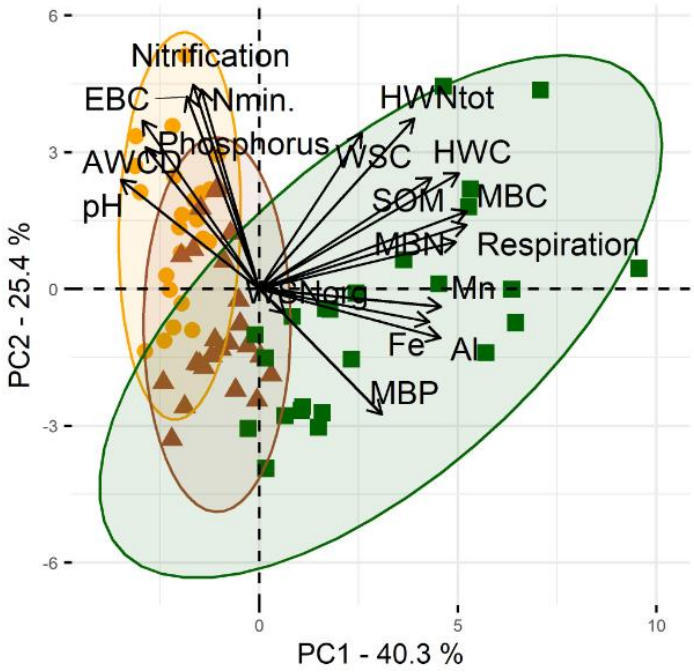

Figure 2.3 Multivariate analyses (PCA) conducted on the soil properties and processes of the soil samples from the potato (●), fallow (▲), and eucalyptus (■) land uses. For each land use the 95% confidence ellipses are shown.

Table 2.3 Results of Linear Mixed-Effects modeling to determine the strength of association between soil properties and soil processes. Models fitted with soil pH_{KCl}, exchangeable base cations (EBC) and either soil organic matter (SOM), hot water carbon (HWC), or microbial biomass carbon (MBC) as explanatory variables (set 1-3). For net N mineralization and net nitrification hot water extractable nitrogen (HWN_{tot}) was also included as explanatory variable (set 4). Four different set of models were thus fitted, and for each set the parameter estimates (Est.) are presented, and the marginal $R^2LMM_{(m)}$, and semi-partial R^2_i were calculated according to Jaeger et al. (2016), (= p-value <0.05; ** = p-value <0.01).*

	Respiration potential		Net nitrogen mineralization		Net nitrification		AWCD	
Set 1: SOM								
$R^2_{LMM(m)}$		0.37		0.31		0.33		0.44
	R^2_i	Est.	R^2_i	Est.	R^2_i	Est.	R^2_i	Est.
SOM	0.29	0.003**	0.17	0.01*	0.18	0.01**	0.01	$-4.8x10^{-04}$
pH_{KCl}	0.01	0.04	0.00	0.05	0.00	-0.05	0.09	0.17*
EBC	0.00	-0.001	0.02	0.04	0.03	0.04	0.09	0.02*
Set 2: HWC								
$R^2_{LMM(m)}$		0.81		0.49		0.50		0.41
	R^2_i	Est.	R^2_i	Est.	R^2_i	Est.	R^2_i	Est.
HWC	0.78	$2.6 x10^{-04}$**	0.40	$7.4 x10^{-04}$*	0.41	$7.1 x10^{-04}$*	0.01	$2.7 x10^{-05}$
pH_{KCl}	0.00	$8.2 x10^{-04}$	0.01	-0.19	0.01	-0.17	0.14	0.19*
EBC	0.00	$6.8 x10^{-04}$	0.03	0.04	0.04	0.04	0.08	0.01*
Set 3: MBC								
$R^2_{LMM(m)}$		0.66		0.17		0.19		0.40
	R^2_i	Est.	R^2_i	Est.	R^2_i	Est.	R^2_i	Est.
MBC	0.61	$5.9 x10^{-04}$**	0.06	$7.2 x10^{-04}$	0.07	$7.4 x10^{-04}$	0.01	$8.3 x10^{-05}$
pH_{KCl}	0.00	$6.5 x10^{-03}$	0.01	-0.22	0.01	-0.20	0.14	0.20**
EBC	0.00	$3.2 x10^{-03}$	0.07	0.07*	0.08	0.07	0.08	0.02*
Set 4: HWN_{tot}								
$R^2_{LMM(m)}$		-		0.47		0.54		-
	-	-	R^2_i	Est.	R^2_i	Est.	-	-
HWNtot	-	-	0.39	0.02**	0.46	0.02**	-	-
pH_{KCl}	-	-	0.04	-0.29	0.04	-0.27	-	-
EBC	-	-	0.01	0.03	0.02	0.02	-	-

2.4 Discussion

2.4.1 Effects of eucalyptus plantations on soil properties and processes

Plantation of eucalyptus on fields previously cultivated with potato lead to an increase in SOM, labile carbon fractions (HWC, MBC), HWC:HWN_{tot}, exchangeable Al, respiration potential, and a decrease in EBC, Nmin, net nitrification, and AWCD. Previous studies also showed an increase in SOM and water extractable C fractions under eucalyptus plantations compared to pasture soils and cultivated areas (Bai and Blumfield, 2015; Kumar et al., 2018). This can be explained by (1) the higher amount of litter returning to the soil under eucalyptus (Chantigny, 2003; van Leeuwen et al., 2017), and (2) lower organic matter oxidation compared to potato and fallow plots, where previous tillage and potato harvesting may have caused aggregate breakdown and increased decomposition (Islam and Weil, 2000). The higher SOM and labile carbon fractions were related to an increase in soil respiration potential, as illustrated by their positive relationships. Our results indicated a higher substrate availability (higher qmic) compared to potato plots, but increased microbial energy maintenance demands (higher qCO_2) indicate lower substrate quality (Anderson & Domsch, 1990; Thirukkumaran & Parkinson, 1999). The reduced metabolic potential of soil bacteria (AWCD) under eucalyptus also indicates lower quality substrate (high content of compounds with low biodegradability, e.g. lignin), despite the increase of labile carbon fractions (SOM, HWC, MBC) under this tree. A shift of the microbial community towards fungal dominance might also have occurred under eucalyptus, as indicated by the decrease in the microbial C:N ratio. However, the magnitude of change was low (mean values: 8.7 under potato, 5.8 under eucalyptus) and within the range where fungal and bacterial C:N ratios overlap (Strickland and Rousk, 2010).

Despite the absence of differences in bulk soil pH_{KCl} between land uses, the fourfold increase in exchangeable Al^{3+} and the higher exchangeable Fe^{2+} and Mn^{2+} under eucalyptus are indicative of soil acidification, possibly due to organic acids secreted by eucalyptus roots (Prosser et al., 1993). Aluminum may reduce crop growth through its phytotoxicity to roots (Al^{3+} and $Al(OH)^{2+}$; Kinraide, 1991), and through a decrease in P availability (Kretzschmar et al., 1991; Robarge and Corey, 1979). The absence of external P inputs may explain the three times lower $NaHCO_3$-extractable P under eucalyptus compared to soils under potato fields. Increased aluminum may also affect soil functions related to N cycling, as N mineralization and nitrification are inhibited by Al through the suppression of enzymatic activities (Kunito et al., 2016; Tietema et al., 1992). Lower C quality, Al toxicity, as well as antimicrobial and allelopathic properties of volatile oils and toxins of excreted by eucalyptus (Cai et al., 2010; Cermelli et al., 2008; Chen et al., 2013) may cause microbial stress (increased energy maintenance demands, qCO2) and contribute to the lower metabolic potential of soil bacteria (AWCD) under this tree compared to potato soils. Altogether, our results indicate profound effects of eucalyptus plantations on soil properties, microbial processes and functions related to C and N cycling, which may be associated to the inability to cultivate potato after eucalyptus, as reported by local farmers (Morales and Patiño, 2008).

2.4.2 Effects of fallowing on soil properties and processes

Fallow and potato land uses were similar for most soil properties, except for the higher WSN_{tot} and extractable P in fallow soils. Higher WSN_{tot} and extractable P in potato cultivated fields can be attributed to mineral and organic fertilization, and subsequent mineralization of organic fertilizers. Our data indicate that the effects of fertilization on extractable P and N decrease over short time periods (2-6 years) after the conversion to fallow soil, confirming the findings of Condori et al. (1997) who showed that the effects of chemical P additions on extractable P lasted for up to two years after fertilization.

Labile fractions such as MBC and MBN have been reported to increase quickly as a result of land-use change from agriculture to grassland (Carter and Rennie, 1982; Landgraf, 2001). Such changes have commonly been associated with fertility restoration, as soils that maintain a high level of microbial biomass are capable of not only storing more nutrients, but also of cycling more nutrients that are easily available to plants (Anderson & Domsch, 1980; Ghani et al., 2003; Joergensen, 2010; Stenberg, 1999). However, we did not find differences in MBC nor MBN between potato and fallow soils, indicating that fallow periods of 2-6 years might not restore soil fertility. In contrast, MBP was higher under fallow soils, despite higher extractable and total P in potato soils. This is consistent with previous research, indicating that $NaHCO_3$-extractable P might not be a good indicator of P availability to plants and microorganisms, as they display adaptive mechanisms enhancing P acquisition from the soil (Brookes et al., 1984; Bucher, 2006; Lambers et al., 2007). Low MBP in potato fields might be due to the higher plant competitiveness and P requirements during tuber formation/growing, resulting in low P availability to soil microorganisms (Alvarez-Sánchez et al., 1999; Castro, 2005). Conversion to fallow would then lead to increased MBP as a result of higher P availability and storage of excess P in microbial cells (Achbergerová and Nahálka, 2011; Heuck et al., 2015). These differences in MBP resulted in changes in the microbial C:N:P molar ratios, with potato soils showing the highest ratios (238:31:1), above the world average for grassland and agricultural soils (60:7:1, Cleveland & Liptzin, 2007; Hartman & Richardson, 2013), indicating potential P limitation to microbial metabolism (Hartman and Richardson, 2013).

We did not find differences in HWN_{tot} or WSN_{org} between land uses, despite the fertilization of potato crops with chicken manure and mineral N. This indicates that these external N inputs do not contribute to total or organic N built up (Condori et al., 1997; Hepperly et al., 2009). The higher net N mineralization and nitrification rates in potato plots are likely due to increased substrate availability from organic fertilization and soil disturbance caused by tillage and harvesting (Li et al., 2001). Higher N turnover would increase mineral N availability for plants and microorganisms (Curtin et al., 2006) but coupled with excessive N fertilization (FAO, 1999), it may also lead to NO_3^- leaching and groundwater contamination. This is also supported by the WSN_{tot} which is mainly composed of NO_3^-. Upon conversion to fallow, net N mineralization and net nitrification rates would depend on the residual fertilizer and on litter returning to the soil from colonizing vegetation.

Studies of long fallow periods (>10 years) have associated fertility restoration with increased total soil C, potentially mineralizable C and N, microbial biomass and cation exchange capacity an (Sarmiento and Bottner, 2002; Sivila and Angulo, 2006). Our results indicate that the short fallow periods currently practiced do not contribute to such changes. As the reduced available land and high food demands do not allow for long fallow periods, alternative sustainable practices are needed to allow high-yield potato cultivation.

2.4.3 Soil carbon fractions as indicators of changes in soil microbial processes

All three carbon fractions assessed in this study (SOM, HWC and MBC) were related to the respiration potential, but HWC was the best predictor, explaining up to 81% of the variation in respiration across land uses. Similarly, (Wang et al., 2003) also found that extractable C fractions were better predictors than soil organic carbon for respiration of rewetted soils. The labile hot water extractable C contains more easily available substrates for microorganisms (Landgraf et al., 2006) and might thus be a better indicator of soil functions related to C and N cycling than SOM and MBC.

HWC was also the best predictor for net N mineralization and net nitrification, with models explaining 49 and 50% of their variation. The lower strength of association in comparison to the respiration potential may be due to the fact that net N mineralization is the result of several gross processes and that nitrification is controlled by autotrophic microorganisms, not directly depending on soil carbon. Also, Colman & Schimel (2013) suggested that the chemical forms of organic nitrogen and their interaction with soil minerals, controlling accessibility of organic nitrogen to microorganisms, as well as potential differences in microbial community composition may be additional controls of net N mineralization. As HWC was correlated to HWN_{tot}, models including HWN_{tot} showed similar explanatory power. As reported previously (Colman and Schimel, 2013; Templer et al., 2003), MBC was not a good predictor of net nitrogen mineralization and nitrification. The low explanatory power of MBC for net nitrification is due to the fact that the nitrifying autotrophic archaea and bacteria are less abundant than heterotrophs and represent only a fraction of total microbial biomass. Previous studies, however, reported a significant correlation between MBC and net N mineralization in forest soils (Malchair and Carnol, 2009), indicating that factors driving net N transformations may vary across ecosystems.

In contrast to respiration and N mineralization, metabolic diversity of soil bacteria was mainly related to soil pH_{KCl}, and to a lower extent, to EBC but not to labile C and N fractions. This differs from findings of Juan et al. (2015), who concluded that SOC availability under organic amendments increased metabolic diversity of soil bacteria. However, the increase of metabolic diversity with soil pH, as also documented by D'Acunto et al. (2018), is in agreement with general microbial theory of positive effects of soil pH on microbial diversity (Wakelin et al., 2008; Willey et al., 2017).

2.5 Conclusions

Soil use and soil fertility have become a crucial issue in the Andean region due to the growing population and land-use intensification. We investigated differences in soil properties and microbial processes related to C and N cycling following the conversion of potato crops to fallow fields and to eucalyptus plantations in agricultural highlands of the Central Andes. The plantation of eucalyptus on soils previously cultivated with potato caused drastic changes: respiration potential increased, while net N transformations, P availability and metabolic diversity of soil bacteria decreased. The traditional long fallow practice has been replaced by 2 - 6 year fallowing periods, which did not result in soil fertility restoration, as it did not lead to major changes in soil properties and microbial processes compared to potato crops. The labile soil carbon fractions were better indicators of soil C and N mineralization processes than SOM. Overall our results show that the use of eucalyptus for afforestation should be considered carefully, especially in communities where agriculture constitutes the main economic income. Also, alternative management practices are needed for potato production in replacement of the long fallowing periods. HWC was the best indicator for soil processes and should be considered for monitoring changes in soil functions related to C and N cycling in response to land-use change.

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Author contributions

Alejandro Coca Salazar: Conceptualization; investigation; formal analysis; writing – original draft (equal), review and editing (equal). Jean-Thomas Cornelis: Conceptualization; writing – review and editing (supporting), funding acquisition; supervision. Monique Carnol: Conceptualization; formal analysis (supporting); writing – original draft (equal); writing – review and editing (equal); resources, funding acquisition, supervision.

Chapter 3

Contribution of soil microbial biomass and oxy-hydroxides to aggregate stability and size distribution under different land uses in the Central Andes

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Abstract

Land use is one of the main drivers disrupting soil structure by changing aggregate stability and size distribution, influencing in turn soil fertility, plant nutrition and food production. Here we evaluated the aggregate size distribution and stability under three land uses in an agricultural region of the Central Andes in Bolivia, the relationships of aggregates with carbon fractions, soil oxy-hydroxides and microbial processes. We found increased amounts of megaaggregates by 4.2 and 2.6 in relation to potato and fallow land uses, indicating improvement of soil structure. Higher C and N contents, and higher values for aggregate stability indexes under eucalyptus plantations were also found, indicating organic matter stabilization. Aggregate stability decreased and the quantity aggregates of <2000 µm increased under potato land use. Results showed that fallow land use did not lead to changes in soil structure compared to potato land use, indicating that this practice does not produce significant changes in soil structure. Alternative management practices are required to maintain soil structure of cultivated and fallow soils. Microbial biomass carbon best explained the variation of the amount of megaaggregates in soil and aggregate stability compared to other C fractions and to soil oxy-hydroxides. Changes in the MBC could be indicative of early changes of the binding agents contributing to the formation organo-mineral complexes. Results also showed that the respiration potential was related to megaaggregates, and that net N mineralization was related to aggregates <250 µm, indicating that higher transformation rates would contribute to aggregate formation. Factors affecting microbial activity would indirectly influence the formation of aggregates.

Keywords: Soil structure, water-stable aggregates, soil oxy-hydroxides, microbial biomass carbon.

3.1 Introduction

Soil structure plays a key role in soil fertility, as it determines soil nutrient and water retention capacity, aeration, and the sequestration of soil organic carbon. A change in soil structure may thus influence plant growth and biomass production by altering the capacity of a soil to provide water and nutrients to plants (Emadi et al., 2009). Land use is one of the main factors driving changes in soil structure, which is commonly assessed through the modifications of aggregate abundance and stability (Rabot et al., 2018). For example, in agricultural land use, tillage may lead to aggregate breakdown, increased proportion of smaller aggregates and reduced stability (Tobiašová et al., 2016; Whalen and Chang, 2002), while organic amendments could promote aggregate formation and stability (Caravaca et al., 2002). In other land uses, such as grasslands or forests, aggregate formation and stability are often related to soil organic matter accumulation and to the increase in the amount of bigger aggregates (Emadi et al., 2009). These changes in soil structure may influence availability of nutrients such as N (Xiao et al., 2019), and therefore affect crop productivity. Consequently, changes in soil structure have been reported to be highly correlated to crops yields (Mueller et al., 2013). In managed ecosystems, evaluation of the changes in soil structure are thus of central importance to determine the potential consequences of changing land uses for soil fertility and food production.

Soil aggregates constitute the basic units of soil structure and their formation is attributed to the interactions between soil organic molecules and minerals (Bronick and Lal, 2005; Oades and Waters, 1991). Commonly, the role of carbon (C) fractions (e.g. total organic C, water extractable C) and soil minerals (silicates and aluminum-, iron-, manganese- oxy-hydroxides) in aggregate formation is inferred from their respective contribution to aggregate size classes and stability (Mustafa et al., 2020; Yang et al., 2013; Doetterl et al. 2015). In soils where organic compounds drive the formation of aggregates, changes in soil organic matter or extractable C fractions have been reported to be correlated to aggregate quantity and stability (Oades and Waters, 1991; Six et al., 2004; Yan et al., 2016), whereas in oxide-rich soils minerals, such as iron, aluminum or manganese oxy-hydroxides have been reported to be correlated with aggregate quantity and stability (Duiker and Rhoton, 2003; Zhao et al., 2017).

Soil microorganisms also play a central role in the formation of aggregates through the binding effect of the microbial-derived organic compounds (Costa et al., 2018; Tang et al., 2011). Aggregate formation thus not only depends on the electrostatic interaction between organic and mineral particles, but also on microbial activity (Crawford et al., 2012; Rabbi et al., 2020). For example, Rabbi et al. (2020) presented evidence that the degradation of carbon substrate by soil microorganism initiates the formation of aggregates, and that their stability is primarily conferred by microbial carbon. However, relatively few information exists on the contribution of the soil microbial C or microbial activity to aggregate stability and size distribution as affected by land use (Al-maliki and Scullion, 2013; Angers et al., 1993). Due to the sensitivity of soil microorganisms to environmental conditions, land-use induced changes of the microbial biomass and microbial activity, such as respiration

potential and nitrogen mineralization, may influence aggregate formation and stability. For example, land-use changes may drastically modify soil organic matter content, nutrient availability, and therefore affect microbial biomass and microbial processes. Changes in microbial biomass and activity would lead to changes in the production of microbial-derived organic compounds that contribute to binding of soil particles (Ekenler and Tabatabai, 2007; Simpson et al., 2004) and ultimately affect the formation of aggregates and their stability. Determining the contribution of the microbial C fraction and microbial processes to soil aggregate stability and size distribution following land-use changes is important for a better understanding the role of soil microorganisms in soil structure, and for predicting the potential consequences of the changes in soil structure.

In the mountainous areas of the Central Andean of Bolivia, agriculture is the main activity involving about 71% of the rural population (INE, 2015). Over the last decades, the growing population and food demand led to intensification of land used for cropping. The traditional practice of leaving arable soil in long fallow periods (>10 y) was thus shortened to 2–6 years. Moreover, eucalyptus plantations were introduced as an alternative economic income to farmers, and replaced previously cultivated areas. Currently the landscapes are dominated by three types of land use: agricultural fields, fallow fields and eucalyptus plantation. Maintenance of soil structure is essential for long-term sustainability of these ecosystems, particularly because increased soil erosion has been associated to land-use intensification in the region (Aalto et al., 2006; Ellis-Jones and Mason, 1999). Despite the fact that soil degradation has been acknowledged as a main problem in agricultural ecosystems of the Andean range (Zimmerer, 1993b), virtually no information exists on the effects of land-use change on soil structure. Determining the contribution of the microbial C fraction and microbial processes to soil aggregate stability and distribution of soil size fractions following land use changes is therefore critical to assess.

In this study we investigated the effects of three land uses (potato agricultural fields, fallow fields and eucalyptus plantations) on soil aggregate stability and size distribution. We hypothesized that eucalyptus plantations would lead to an improvement in soil structure, while potato and fallow land uses would be similar due to intensified cultivation and short fallow periods. Further, we aimed to understand which soil constituents (C fractions, and soil oxy-hydroxides) contributed to aggregate stability and size distribution under the three land uses. We hypothesized that soil C fractions would be better indicators of the changes in aggregate stability and size distribution compared to soil oxy-hydroxides. Finally, we aimed at determining the link between microbial activity, measured through respiration potential and N mineralization, with aggregate size distribution, and hypothesized that higher microbial transformation rates would lead to increases in the amount of larger aggregates and aggregate stability.

3.2 Materials and methods

3.2.1 Study area and soil sampling

The study was conducted in an agricultural area of Cochabamba-Bolivia located in the Eastern branch of the Central Andes range (17°32'30" - 17°33'30" S, 065°20'08" - 065°21'36" W, between 3100 and 3400 m altitude). Climate is characterized by a rainy summer season (November-March) and a dry winter season (April-October), with a mean annual rainfall of 500.7 mm, and a mean annual temperature of 17.9 °C (Navarro and Maldonado, 2002; SENAMHI, 2016). The topography is characterized by secondary mountain ranges of the Andean region, with soils classified as Cambisols (Ministerio de Medio Ambiente y Agua, 2014).

Potato crops, fallow fields and eucalyptus plantations are interspersed within the landscape in areas of ca. 0.5 - 1 ha, defined here as "plots". Eight plots of each land use type were selected within an area of ca. 4 km^2 based on the following criteria: agricultural plots in which potato had been grown during the last rainy season, fallow plots (2-6 years old) in which the spontaneously grown vegetation (grass-shrubland of semiarid high Andes; Navarro and Maldonado, 2002) fully covered the soil, and Eucalyptus (*E. globulus)* plantations of minimum 5 years since plantation. Fields cultivated with potato are plowed before cultivation and potato harvesting is conducted manually. Before conversion to fallow, remaining aboveground biomass is incorporated by tillage and the fields are then left unmanaged. Similarly, eucalyptus plantations are not managed after seedling plantation. At the end of the rainy season (February 2017), soils were sampled 2-3 weeks after harvesting took place in the potato plots. One composite soil sample (one central sample and four samples 2 m around) was taken in each plot with a shovel at 20 cm depth at a randomly selected point. In order to work on mineral soils for each studied site, the thin forest floor layer (<0.5 cm) of the eucalyptus plots was discarded. All samples were analyzed for soil aggregate size classes.

3.2.2 Soil aggregate size classes and quantification of oxy-hydroxides

Soil aggregate size fractionation was performed through the wet sieving method (Elliott, 1986; Six et al., 1998) which allowed the isolation of the megaaggregates (>2000 µm), macroaggregates (250–2000 µm), free microaggregates (53–250 µm), free silt+clay (<53 µm), occluded microaggregates (53–250 µm, aggregates contained within macroaggregates) and occluded silt+clay (<53 µm particles contained within macroaggregates). Briefly, sixty grams of soil were placed on a 2000 µm sieve submerged in distilled water for 5 min at room temperature. Wet sieving was conducted by moving the sieve out of the water and immersing it again 50 times over 2 minutes. The remaining water and particles that passed through the sieve were used to repeat the process using the 250 µm and 53 µm sieves. The particles retained by the sieves were recovered, oven-dried at 50°C and weighted. This allowed the isolation of the >2000 µm (megaaggregates), 250–2000 µm (macroaggregates), and 53–250

µm (free microaggregates) size classes. The remaining water was oven-dried at 50°C to recover the <53 µm (free silt+clay) size class.

The occluded 53–250 µm size class (occluded microaggregates) was mechanically separated from the macroaggregates (Six et al., 2000). Briefly, 10 g macroaggregates were suspended in 50 ml distilled water and left overnight. The solution was poured on top of a 250 µm sieve and shaken with 60 glass beads (3 mm diameter) for 15–20 min at 250 rpm under a continuous flow of distilled water. The solution was then passed through a 53 µm sieve at the outlet of the system to recover the occluded microaggregates which were oven-dried at 50 °C and weighted. The occluded silt+clay size class was calculated by subtracting the weight of the occluded microaggregates and the weight of the particles retained in the 250 µm mesh from the total weight of macroaggregates used for isolation of occluded microaggregates.

Given that there is little or no binding of organic matter with sand particles (Elliott et al., 1991), the weight of isolated size classes was corrected with the sand contents of the respective size class by subtracting the weight of the sand contained in the mega, macro and microaggregates. The percentages of sand content in the mega and microaggregates were determined by dispersing 3–5 g of each size class in 15 ml 5% sodium hexametaphosphate solution. After overnight agitation the solutions were passed through 2000 and 53 µm sieves respectively, and the particles remaining in the sieves were dried, weighted and used to calculate the percentage of sand content. The weight of the sand particles retained in the 250 µm mesh during occluded microaggregates isolation was used to calculate the percentage of sand content in the macroaggregates.

The percentage of total water-stable aggregates (WSA) and the mean weight diameter (MWD) were used as indicators of aggregate stability (Yan et al., 2016). WSA is the percentage of aggregates retained in the sieves >53 µm relative to the total weight of the soil sample sieved, and the mean weight diameter (MWD) was calculated as:

$$MWD = \sum_{i=0}^{n} X_i \times \mathrm{W}_i \quad (1)$$

where n is the number of aggregate size classes (n= 4, with the classes being mega, macro, microaggregates and silt+clay), X_i is the mean diameter of the isolated aggregate size class i (with the mean diameters calculated as 3.5, 1.125, 0.1515, 0.053 mm for the mega, macro, microaggregates and silt+clay size classes, respectively; Yan et al., 2016), and W_i is the weight of the aggregate size class i.

The concentration of total organic carbon (TOC) and total nitrogen (TN) in the mega, macro, microaggregates, occluded microaggregates and free silt+clay size classes were measured by flash-dry combustion (TruMac CN analyzer, LECO), and the data was used to calculate the C:N ratios of each aggregate size class. Carbon and nitrogen contents of each aggregate size class per kilogram of bulk soil were calculated using the TOC and TN concentrations

and the amount of each aggregate size class in the soil. We assumed that the TOC and TN concentrations in the free silt+clay class was the same as the concentration in the occluded silt+clay class for its C and N content calculation (de Tombeur et al., 2018).

The free (crystalline and amorphous) Fe, Al, Mn oxy-hydroxides were measured in bulk soil samples using the dithionite-citrate sodium bicarbonate (DCB) method (Mehra and Jackson, 1958), and the amorphous forms of oxy-hydroxides were extracted with the ammonium oxalate method (Schwertmann, 1964). The DCB-extractable Fe, Al and Mn were quantified from 1 g soil mixed with 40 ml sodium citrate 0.3 M and sodium bicarbonate 0.25 M solution on a water bath at 75°C and mixed with 3 g of sodium dithionite. The solution was centrifuged (10 min, 2000 rpm), poured to a 250 ml, and the remaining soil was rinsed with 25 ml NaCl until the red-brown color disappeared. The oxalate-extractable Fe, Al and Mn were extracted from 1 g soil agitated with 100 ml of an oxalic acid-ammonium oxalate solution for 4 h in the dark and filtered with Whatman 602 h ½. The DCB (Fe_{DCB}, Al_{DCB}, and Mn_{DCB}) and oxalate (Fe_{Ox}, Al_{Ox}, and Mn_{Ox}) extractable elements were measured by atomic absorption spectrometry (Varian)

3.2.3 Bulk soil C fractions and biological characterization

Total soil organic carbon (TOC), total nitrogen (TN), water soluble carbon (WSC), hot water extractable carbon (HWC), microbial biomass C (MBC), and the microbial variables CO_2 respiration potential and net N mineralization were measured in bulk soil. TOC and TN were measured with flash dry combustion on 0.5 g soil. WSC and HWC were determined by measuring the total organic carbon in cold and hot water extracts according to Ghani et al. (2003). MBC was determined as the difference of K_2SO_4 extractable C between chloroform fumigated and non-fumigated soil samples. Respiration potential was calculated as the CO_2-C increase over a 3 hour incubation of 20 g fresh soil (Robertson et al., 1999). Net N mineralization is the increase in the total mineral N after a 28 day incubation period (20°C and constant humidity; Hart et al., 1994). Detailed methods and land-use effects on WSC, HWC, MBC and microbial processes can be found in Coca-Salazar et al. (2021).

3.2.4 Statistical analyses

Differences between the amounts of aggregates in size classes, their TOC, TN contents, stocks and Fe, Al, Mn oxy-hydroxides between land uses were assessed with ANOVA and post-hoc Tukey tests. Differences between the amounts of aggregates size classes, their TOC, TN contents within each land use were assessed with ANOVA and post-hoc Tuckey test. When heterogeneity of variances was detected, Welch ANOVA and Games-Howell post hoc tests were used (Faria et al., 2018; Mangiafico, 2015).

Redundancy analyses (RDA) was used to explore the relationships between MWD, WSA and amounts of aggregate size classes (dependent variables) with the contents of soil C fractions (TOC, WSC, HWC, MBC), and oxy-hydroxides content (explanatory variables) according to Borcard et al. (2018). A full model including all dependent and explanatory variables was constructed (centered data), and an optimal model was selected through

backward selection process by removing explanatory variables with variance inflation factors (VIF) higher than 10, and by comparing the Akaike information criteria (AIC) of models. The significance of the optimal model and the variables were tested through permutation tests (significance level of 0.05). The variance explained by the explanatory variables of the optimal model was also calculated (variance partitioning of the RDA model). To corroborate the association between the variables of the optimal RDA model we performed least square linear regressions between the amount of soil aggregate size factions, MWD and WSA as response variables, and MBC and Fe and Mn extracts as explanatory variables.

The covariation between changes in the amount of aggregates of different sizes and soil processes (respiration potential, net N mineralization) was assessed through multivariate co-inertia analyses (Dray et al., 2003). The co-inertia analyses (COIA) was conducted with the following datasets: (1) amounts of aggregates in size classes, and their (2) TOC and (3) TN contents; with (4) microbial processes (respiration potential and net N mineralization). First, we performed principal component analyses (PCA) on the above mentioned centered datasets to calculate the total inertia for each (Stephane Dray et al., 2003; Li et al., 2009), then COIA were computed between pairs of PCAs. Monte Carlo permutation tests were conducted on the sum of eigenvalues of each COIA, to test the significance of the correlation coefficients between the two data sets used (RV coefficient). To corroborate the association of the variables that co-varied, we performed least square linear regression analyses between amount of soil aggregates in size factions, their C and N contents as response variables, and microbial processes as explanatory variables.

Statistical analyses and graphs were made with the R software (R Core Team 2019) using the packages “multcomp” (Hothorn et al., 2017), “multcompView” (Graves et al., 2015), “ade4” (Dray et al., 2018), and “adegraphics” (Siberchicot et al., 2017), “factoextra” (Kassambara and Mundt, 2019), “vegan” (Oksanen et al., 2019).

3.3 Results

3.3.1 Soil aggregate size classes and oxy-hydroxides

The recovery of soil particles from wet sieving was almost complete, ranging from 98 to 100% of the soil used. Under eucalyptus, aggregate size distribution was dominated by the amount of mega and macroaggregates with significantly higher values (p-value <0.05) compared to the other size classes, in the following order: megaaggregates (37.7%), macroaggregates (32.6%), occluded silt+clay class (16.1%), and the other size classes (<8%) (Table 3.1). In contrast, macroaggregates were most abundant under potato and fallow soils (46.5 and 49.6%, respectively, p-value <0.05) compared to smaller aggregate sizes, followed by the occluded silt+clay (23.7% and 28.0%, respectively), the free silt+clay (17.5% and 15.5%, respectively), and remaining aggregate sizes (<10%).

Land-use change led to clear differences in aggregate size distribution and stability (Table 3.1). Eucalyptus land use led to significantly higher MWD, WSA, total aggregate, and

megaaggregate content in comparison to potato and fallow land uses, while the quantity of macroaggregates, occluded microaggregates, free and occluded silt+clay were significantly lower under eucalyptus in comparison to potato and fallow land uses (p-value<0.05). The free microaggregates were lowest under eucalyptus, followed by fallow, and highest under potato with significant differences between the three land uses (p-value <0.05).

Table 3.1 Mean weight diameter (MWD), water stable aggregates (WSA), and aggregate size distribution (g aggregate kg kg^{-1} bulk soil) under potato, fallow and eucalyptus land uses (mean ± standard deviation). Different lowercase letters indicate significant differences between land uses, and different uppercase letters indicate significant differences between aggregate size quantities within each land use (p-value <0.05, ANOVA and Tukey tests, n=8).

	Potato	Fallow	Eucalyptus
MWD (mm)	0.83±0.01 b	0.96±0.16 b	1.36±0.01 a
WSA (%)	80.08±5.79 b	82.49±7.34 b	91.35±2.44 a
Total aggregates	707.78±70.17 b	740.35±87.79 b	762.99±50.55 a
Megaaggregates	88.20±28.66 a D	142.30±55.39 a C	377.17±81.87 b A
Macroaggregates	464.72±44.69 a A	496.10±67.42 a A	325.68±61.13 b A
Free microaggregates	154.86±26.50 a DB	101.95±37.62 b C	60.13±14.48 c C
Free silt+clay	175.14±49.27 a BC	155.39±61.37 a C	72.41±22.52 b C
Occluded microaggregates	134.38±36.81 a DB	119.99±22.72 a C	62.75±22.33 b C
Occluded silt+clay	236.63±68.36 a C	279.87±63.85 a B	160.85±41.38 b B

Average TOC recovery within aggregates was 86, 89 and 97%, while average TN recovery was 92, 94 and 96% in in eucalyptus, fallow and potato land uses, respectively. Total organic carbon concentration was evenly distributed across size classes within each land use (Table 3.2). Similarly, total nitrogen concentration in aggregates did not show significant differences in potato land use. The free silt+clay had the highest TN concentrations in fallow and eucalyptus land uses compared to the other aggregate sizes. Comparison of the aggregates' TOC and TN concentrations between land uses showed significantly higher values in the occluded silt+clay class under eucalyptus compared to potato and fallow land uses, while no differences were shown for other aggregate size classes (Table 3.2). Comparison of the C:N ratios within land uses indicated that free silt+clay had significantly lower C:N ratios compared to the other aggregates size classes, while comparison between land uses indicated that the macroaggregates, free microaggregates and occluded microaggregates had significantly higher C:N ratios in eucalyptus compared to potato and fallow land uses.

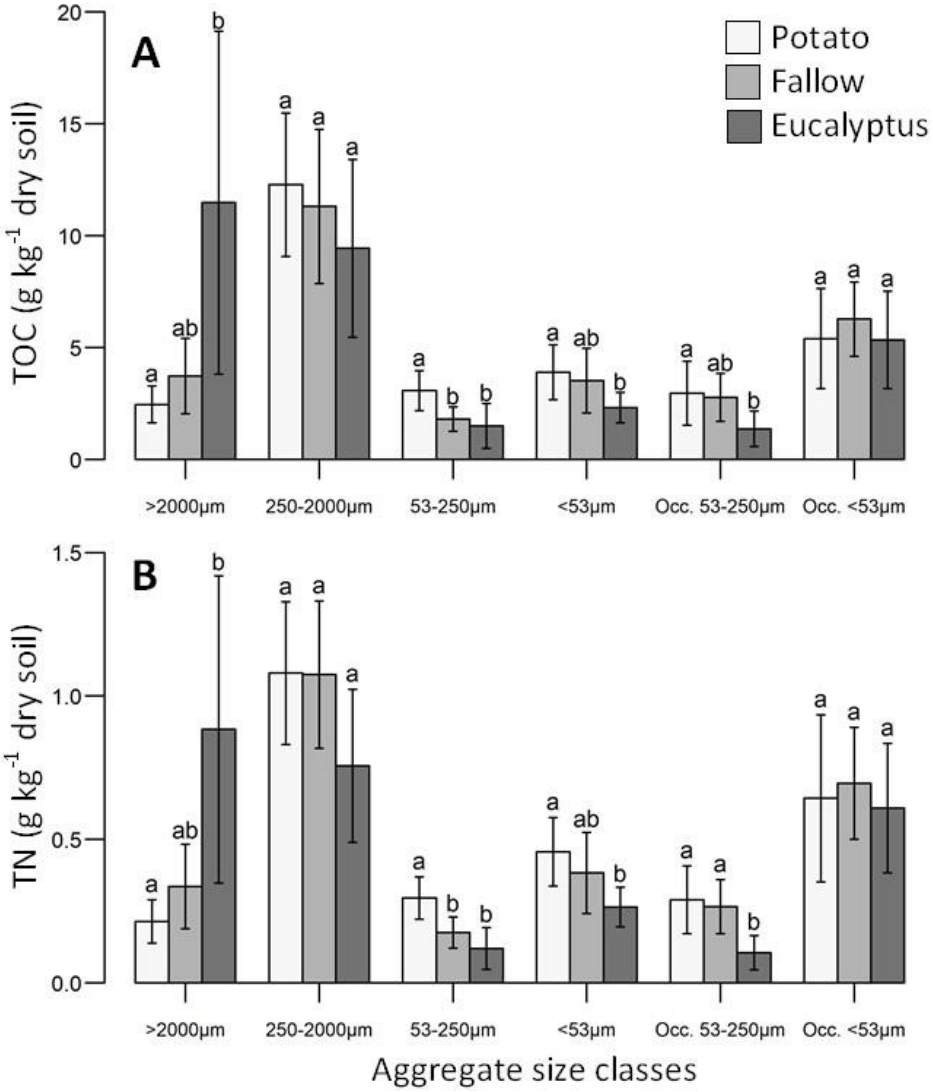

Figure 3.1. Total organic carbon (TOC) and total nitrogen (TN) contribution of each aggregate class to total soil mass under potato, fallow and eucalyptus land uses (mean ± std). Megaaggregates (>2000µm), macroaggregates (250-2000µm), free microaggregates (53-250µm), free silt+clay fraction (<53µm), occluded microaggregates (Occ. 53-250µm), and occluded silt+clay fraction (Occ. <53µm). Different letters indicate significant differences between land uses (p-value <0.05, ANOVA and Tukey tests, n=8).

The TOC and TN content in aggregates relative to bulk soil mass showed significant differences between land uses for most of the size classes (Figure 3.1). Patterns were similar for carbon and nitrogen, with, on average, 4.7 and 3.1 times (carbon) and 4.1 and 2.5 times (nitrogen) significantly higher amounts in the megaaggregates under eucalyptus compared to potato and fallow. For the free and occluded microaggregates, as well as the free silt+clay, values were significantly lower under eucalyptus compared to potato and fallow land uses. The macroaggregate and occluded silt+clay did not show significant differences between land uses for their amounts of C and N.

The analysis of soil oxy-hydroxides indicated that Fe_{DCB} was the most abundant oxy-hydroxide ranging from 2.3 to 24.7 mg g^{-1}, with eucalyptus and fallow fields showing significantly higher values than potato fields (Table 3.3). Al_{DCB} oxy-hydroxides ranged from 0.5 to 5.1 mg g^{-1} without significant differences between land uses, whereas the Mn_{DCB} ranged from 0.2 to 1.2 mg g^{-1} with significant lower values under eucalyptus compared to fallow soils. The Fe_{Ox} ranged between 1.5 to 5.1 mg g^{-1} and the Al_{Ox} ranged between 0.1 to 3.8 mg g^{-1}, both without differences between land uses. The Mn_{Ox} ranged between 0.2 to 1.2 mg g^{-1} with significantly higher values in fallow compared with eucalyptus plots, while potato had intermediate values.

Table 3.2 Total organic carbon, total nitrogen concentrations and C:N ratio of bulk soil and sand-free aggregate sizes under potato, fallow and eucalyptus land uses (mean ± std). Different lowercase letters indicate significant differences between land uses and different uppercase letters indicate significant differences between aggregate size quantities within each land use (p-value <0.05, ANOVA and Tukey tests, n=8).

Aggregate size classes	TOC (g C kg^{-1} sand free aggregates)			TN (g N kg^{-1} sand free aggregates)			C:N ratio		
	Potato	Fallow	Eucalyptus	Potato	Fallow	Eucalyptus	Potato	Fallow	Eucalyptus
Bulk soil	23.57±6.95 a	22.90±5.40 a	28.55±12.47 a	2.16±0.55 a	2.10±0.35 a	2.20±0.84 a	10.80±0.97 b	10.80±1.09 b	12.72±1.04 a
Megaaggregates	27.7±3.3 a A	26.1±5.4 a A	28.8±15.8 a A	0.2±0.0 a A	0.2±0.04 a AB	0.2±0.1 a B	11.5±0.8 a A	11.0±1.0 a A	12.5±2.6 a A
Macroaggregates	26.6±6.9 a A	22.6±5.6 a A	29.6±13.7 a A	0.2±0.1 a A	0.2±0.0 a AB	0.2±0.1 a B	11.3±1.4 ab A	10.7±0.9 b AB	12.1±1.5 a A
Free microaggregates	20.1±5.6 a A	18.5±5.1 a A	24.2±12.5 a A	0.2±0.0 a A	0.2±0.0 a B	0.2±0.1 a B	10.3±0.8 b A	10.4±1.1 b AB	12.2±0.9 a A
Free silt+clay	23.1±7.6 a A	23.0±5.7 a A	33.8±11.1 b A	0.3±0.1 a A	0.3±0.0 a A	0.4±0.1 b A	8.5±0.7 a B	9.1±1.1 a B	8.7±1.1 a B
Occluded microaggregates	22.0±8.5 a A	22.9±6.2 a A	21.9±11.3 a A	0.2±0.1 a A	0.2±0.1 a AB	0.2±0.1 a B	10.1±1.6 b A	10.4±0.9 b AB	12.8±1.2 a A

Table 3.3 DCB and oxalate extractable Fe, Al and Mn oxy-hydroxides under potato, fallow and eucalyptus land uses (mean ± standard deviation; mg kg^{-1} dry soil). Different letters indicate significant differences between land use (p-value <0.05, ANOVA and Tukey tests, n=8).

		Potato	Fallow	Eucalyptus
Fe	DCB	3.66±0.98 b	20.72±3.11 a	18.09±4.08 a
	Oxalate	3.14±1.01 a	3.04±0.48 a	2.48±0.79 a
Al	DCB	3.35±1.32 a	2.27±1.00 a	2.68±1.36 a
	Oxalate	1.23±0.76 a	0.81±0.65 a	1.51±1.07 a
Mn	DCB	0.78±0.23 ab	0.90±0.28 b	0.47±0.31 a
	Oxalate	0.68±0.18 ab	0.77±0.24 b	0.41±0.32 a

3.3.2 Relation between aggregate size classes and stability with binding agents

The selected RDA model explained 58 % of the variation in aggregate stability and size distribution. MBC, Fe_{DCB}, Fe_{Ox} and Mn_{Ox} were the most important soil constituents explaining the variation of the aggregate stability and size distribution. Variance partitioning showed that MBC explained 21%, the soil oxy-hydroxides Fe_{DCB}, Fe_{Ox} and Mn_{Ox} explained 16 %, and the interaction of MBC with Fe_{DCB}, Fe_{Ox} and Mn_{Ox} accounted for 21 % of the variation (Figure 3.2A). Multivariate ordination of the RDA model showed a clear separation of eucalyptus from potato and fallow soils (Figure 3.2B). The estimated data points for eucalyptus were associated with higher values of MWD, WSA, megaaggregates, MBC and $Fe_{DCB,}$ while the estimated data points for potato and fallow soils overlapped and were associated with higher values of macroaggregates, microaggregates, silt+clay and the Mn_{Ox} and Fe_{Ox} oxyhydroxides. Regression analyses of individual variables corroborated these results, with MBC and Fe_{DCB} showing positive correlations with the amount of megaaggregates, MWD and WSA, and the Fe_{Ox} and Mn_{Ox} showing correlations with <2000 µm aggregates (S1 of supplementary material).

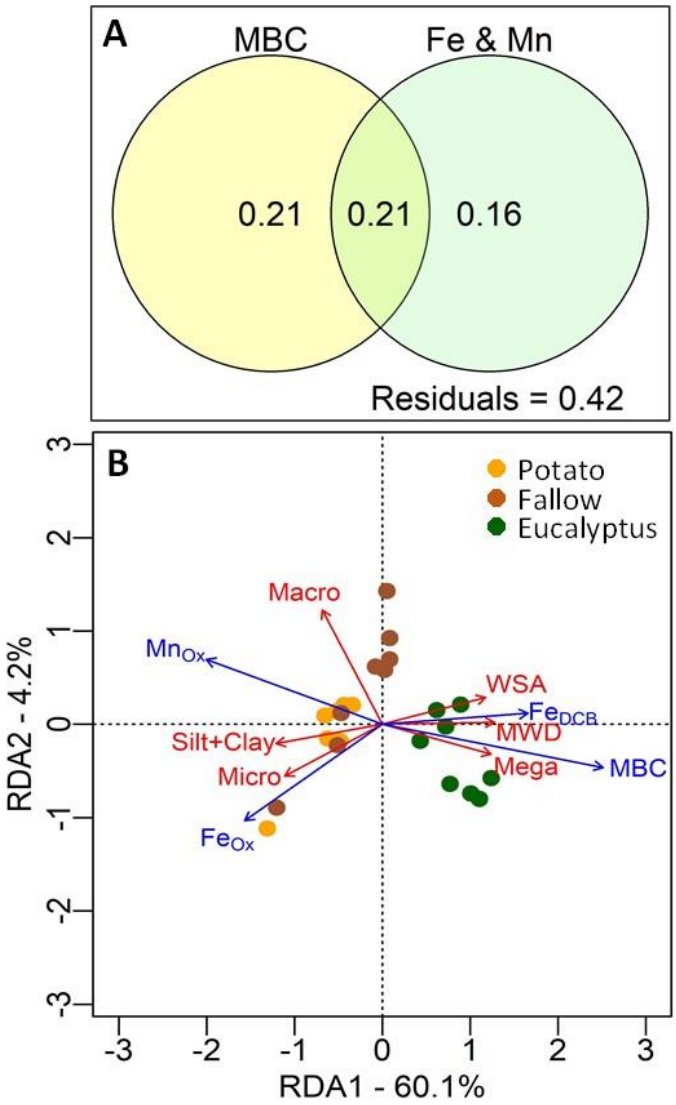

Figure 3.2 Redundancy analyses between mineral binding agents with aggregate size distribution and stability under potato, fallow and eucalyptus land uses. A: ordination plot of the redundancy analyses conducted on the aggregate variables mean weight diameter (MWD), water stable aggregates (WSA), megaaggregates, macroaggregates, microaggregates and silt+clay fractions, with microbial biomass carbon (MBC), Fe_{DCB}, Fe_{Ox} and Mn_{Ox} oxyhydroxides. B: variance in the aggregate variables explained by MBC, and oxy-hydroxides (Fe_{DCB}, Fe_{Ox} and Mn_{Ox}).

3.3.3 Relationship between microbial processes with aggregates and C N contents

Co-inertia (COIA) analyses between the respiration potential and net N mineralization with the amount of aggregates in size classes and their TOC and TN content relative to bulk soil indicated significant co-variation between datasets. The coefficients of correlation (RV coefficient) were 0.53 (Figure 3.3A), 0.51 (Figure 3.3C), and 0.60 (Figure 3.3E), for the amount of aggregates, the TOC and TN contents (p-value <0.05), respectively. Multivariate ordination discriminated eucalyptus from potato and fallow land uses along the main projected axis in the three COIA analyses, which accounted for 74 to 99 % of the total projected co-inertias. Estimated data points for potato and fallow overlapped.

The association of individual variables showed positive co-variation of respiration potential with the amount of megaaggregates and their TOC and TN contents (Figures 3.3B, 3.3D, 3.3F), which was corroborated by the regression analyses (R^2 = 0.75, 0.77 and 0.89, respectively, *p*-value < 0.01, S2 and S3 of supplementary material). COIA results indicated co-variation of net N mineralization with the amount of occluded silt+clay and free microaggregates, but regression analyses indicated a weak correlation with free microaggregates only (R^2 = 0.17, *p*-value = 0.03, S2 of supplementary material). Net nitrogen mineralization also co-varied with TOC and TN contents of macro and free microaggregates (Figures 3.3D, 3.3F). Regression analyses corroborated these results, with free microaggregate TOC and TN contents showing the strongest associations (R^2 = 0.42 and 0.43, respectively, *p*-value <0.05, S2 of supplementary material), relative to macroaggregate TOC and TN contents (R^2=0.14 and 0.16, respectively, *p*-value <0.05, S3 of supplementary material). Regression analyses also indicated positive relationships of net N mineralization with the free silt+clay, occluded microaggregates and occluded silt+clay size classes (R^2 = 0.15, 0.24 and 0.13, respectively, *p*-value <0.05, Table 2 of supplementary material) despite the fact that COIA did not show co-variation of these variables.

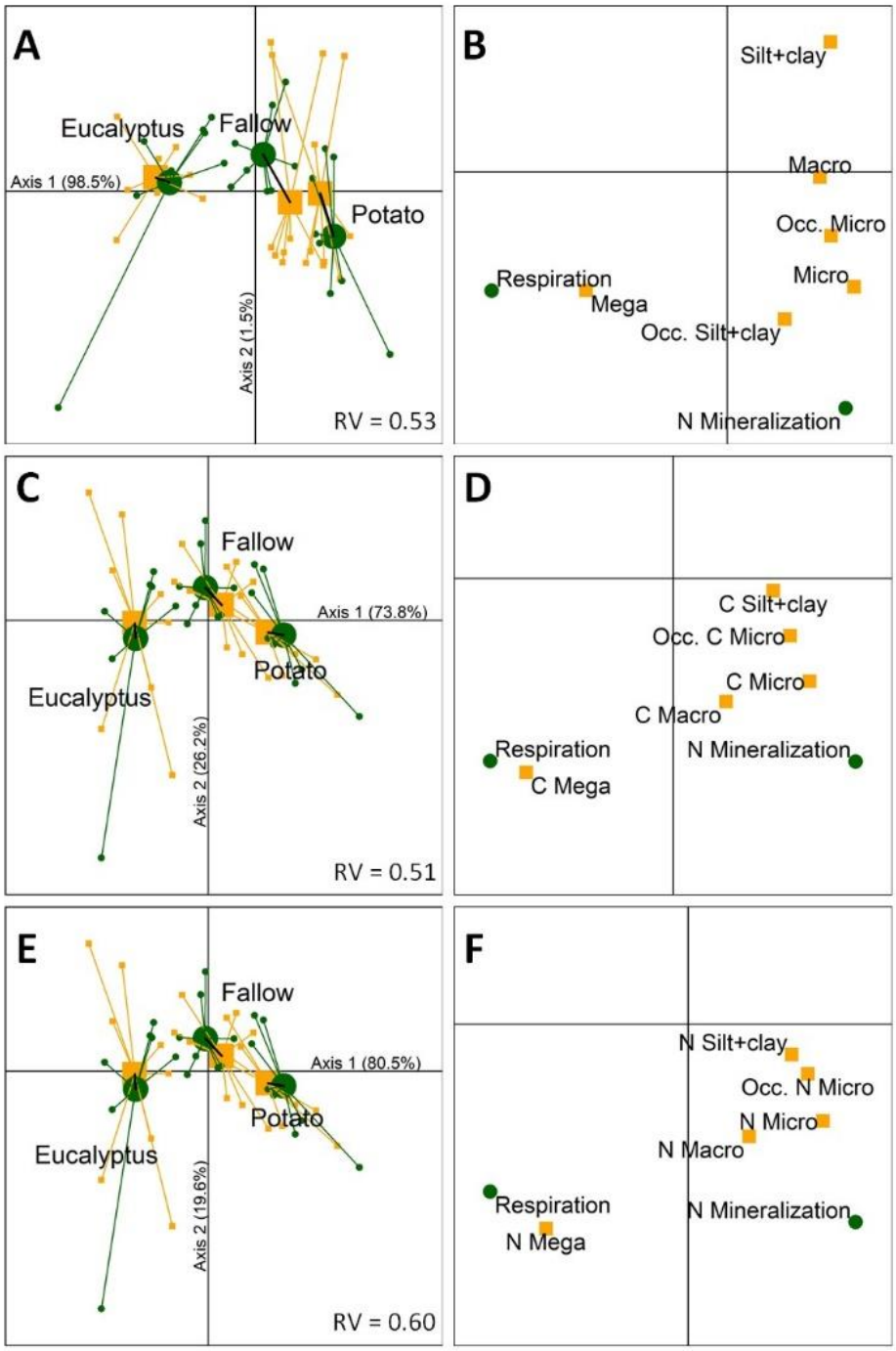

Figure 3.3 Co-inertia analyses of the soil processes respiration potential and net N mineralization with aggregate quantity (A, B), TOC (C, D) and TN (E, F) contents relative to bulk soil. Plots on the left are the discriminant co-inertia analyses, where orange squares represent the estimated values of the aggregates data sets and the green circles represent the estimated values of the soil processes data set. RV = correlation coefficient between the two data sets used in each co-inertia analyses. The biggest squares and circles are barycenters for each land use. Plots on the right are the canonical weights of the variables, where proximity of the variables indicate co-variation of the aggregates (squares) and soil processes (circles).

3.4 Discussion

3.4.1 Land-use effects on soil aggregates, TOC, TN concentrations and contents

In the present study, eucalyptus plantations led to an increase in the formation of megaaggregates at the expense of macroaggregates, microaggregates and silt+clay, compared to soils under potato and fallow land uses. This is likely due to the higher amount of organic matter entering to the soil (litter and root exudates), and the absence of soil physical perturbation (tillage and potato harvesting) that promoted the formation of new aggregates and the binding of the already existing free macroaggregates, microaggregates and silt+clay around fresh plant material (Tisdall and Oades, 1982; Tobiašová et al., 2016). The observed higher soil aggregate stability under eucalyptus is considered to be a result of successful afforestation (Caravaca et al., 2002) and it is generally associated with higher soil C content (Wei et al., 2006). Concomitantly, the higher TOC and TN concentrations in the free silt+clay soil fraction under eucalyptus is in accordance with previous studies suggesting that greater C and N concentrations in this aggregate size are indicative of physical protection and stabilization of soil organic matter (Del Galdo et al., 2003; Six et al., 2000; Tobiašová et al., 2016; Wei et al., 2006). Stabilization under eucalyptus would be the result of slower aggregate turnover allowing the formation of organo-mineral associations (Del Galdo et al., 2003; Denef et al., 2004; Six et al., 2000, 1998), resulting in less accessible organic carbon for decomposition by soil microorganisms (Six et al., 1999; Tobiašová et al., 2016). Despite the positive effect of the plantation of eucalyptus on soil structure, its introduction to this agricultural ecosystem had negative effects on soil microbial activity (net N mineralization, potential nitrification, and metabolic potential of soil bacteria (Coca-Salazar et al., 2021b, 2021a).

Under potato crops, soil management practices such as tillage and potato harvesting lead to reduced aggregate stability and increased megaaggregate breakdown, resulting in an increased amounts of macroaggregates, microaggregates and silt+clay. In particular, the amount of macroaggregates was 1.4 times higher than under eucalyptus. These results are in accordance with previous studies showing reduced stability of megaaggregates due to soil mechanical disruption (Chan et al., 2002; Wei et al., 2006). Increased amounts of macroaggregates, microaggregates, and silt+clay under potato may be also linked to the dispersive effects of N fertilization on soil aggregates (Yan et al., 2016), as potato fields receive chemical and organic N fertilization during the cropping cycles. The increase of the macroaggregates has been linked to the deterioration of soil structure (Tobiašová et al., 2016; Whalen and Chang, 2002), which would increase the risk of soil erosion (Pagliai et al., 2004; Wei et al., 2006). Given the characteristic mountainous topography, negative consequences of reduced aggregate stability such as increased soil erosion, the runoff of soil particles and associated nutrients are highly probable, as reported in similar potato agricultural systems in the North Andean region (Otero et al., 2011).

The effect of soil agricultural practices on aggregate size distribution seems to persist after fallowing potato plots for 2–6 y, since no differences were observed between fallow and

potato soils. Thus, the current short fallow periods (<6 y) would not allow an improvement of soil structure. However, fallowing soils may still contribute to reducing the risk of soil and nutrient loss by runoff due to the protective effects of vegetation colonizing the fields during fallow periods (Otero et al., 2011; Sims et al., 1999).

3.4.2 Contribution of soil constituents to soil aggregation

The role of soil organic matter in the formation and stabilization of soil aggregates is well established (Tisdall and Oades, 1982). The increasing evidence of the central role of soil microorganisms in aggregate formation (Rabbi et al., 2020) supports the need to investigate the contribution of the microbial biomass (MBC) in soil aggregate size distribution (Al-maliki and Scullion, 2013; Angers et al., 1993). Our results suggest that the measured soil properties contribute differently to the stability and size distribution of soil aggregates. MBC and Fe_{DCB} would contribute mainly to megaaggregate formation and stability, while the oxalate-extractable Fe and Mn would be more important for the macroaggregates, microaggregates, and silt+clay.

MBC explained most of the variation of megaaggregate and aggregates stability, which is corroborated by its strong relationships with megaaggregate quantity, MWD and WSA (R^2 = 0.77, 0.72, and 0.60, respectively). Soil microorganisms contribute to aggregate formation through the binding effect of extracellular polymeric substances and the bonding effect of hyphae (Degens, 1997; Six et al., 2006; Tang et al., 2011). Particularly, soil fungi may play a crucial role in aggregate formation, and they have been recognized as important drivers of the recovery of aggregate stability in fallow soils in the Bolivian Altiplano (Duchicela et al., 2013). Management practices contributing to buildup of the microbial C fraction in the upper soil layers (e.g. reduced or no-tillage cultivation; Mathew et al., 2012; Meyer et al., 1996) could contribute to improvement of soil structure and reduce the risk of erosion.

Dithionite extractable Fe contributed to megaaggregates and aggregate stability as indicated by the RDA results and corroborated by its positive association with megaaggregates, MWD and WSA. Electrostatic binding of oxy-hydroxides with negatively charged clay minerals and sorption to soil organic matter compounds may explain these results. However, it is also possible that the relationships could be due to positive correlations between labile C fractions and Fe oxides rather than a direct contribution of Fe_{DCB} to megaaggregate formation. For example, in mountainous meadow ecosystems Li et al. (2017) reported strong correlation between labile C and amorphous Fe oxy-hydroxides, attributed to association through encapsulation of organic compounds in Fe nodules (Xue et al., 2019). The relation of Fe_{DCB} with indicators of aggregate stability and megaaggregate content could be also due to field-scale spatial variability of Fe oxy-hydroxides contents (Santos et al., 2015).

The correlation of Fe_{Ox} and Mn_{Ox} with the <2000 µm aggregates (macroaggregates, microaggregates, and silt+clay) stresses their role in the formation of the smaller aggregate sizes, and in organic matter stabilization through the formation of organo-metallic complexes (Jiménez et al., 2011; Wagai and Mayer, 2007). The positive relationships of Mn oxy-hydroxides with the free and occluded silt+clay sizes (R^2 = 0.33 and 0.40, respectively)

indicated the presence of Mn oxy-hydroxide minerals in fine-grained aggregates with large surface areas such as the silt and clay particles (Post, 1999). Despite the fact that that Al oxy-hydroxides have been also reported to contribute to soil aggregate formation (Barral et al., 1998; Rampazzo et al., 1999), we did not find evidence that Al forms play a major role in aggregate stability and size distribution of these soils.

The interaction between MBC with Fe and Mn oxy-hydroxides explained 21 % of the variation of aggregate stability and size distribution. This may be explained by the formation of abiotic aggregates between soil oxy-hydroxides (e.g. montmorillonite and goethite) due to electrostatic interactions and subsequent accumulation of organic compounds and microorganisms that would lead to microaggregate formation (Krause et al., 2019). Moreover, the adsorption affinity of bacterial cells with minerals such as crystalline goethite would lead to the formation of "clay sized minerals hutches" around bacterial cells, which would act as nucleus for microaggregate formation (Krause et al., 2019; Lünsdorf et al., 2000). Given that soil microorganisms are more sensitive to environmental changes than soil mineralogy, changes in MBC are more likely to take place upon land-use changes. Consequently, changes in the MBC could indirectly influence the formation of aggregates and be indicative of early changes of the binding agents contributing to the formation organo-mineral complexes.

3.4.3 Contribution of soil microbial processes to aggregate formation

The majority of recent studies linking soil aggregation and microbial variables report measurements of soil microbial properties and processes in aggregate size classes isolated with the wet sieving methodology (Han et al., 2019; Li et al., 2020; Tian et al., 2019; Vázquez et al., 2020; Xiao et al., 2017). Wet sieving is considered to be the most representative evaluation of the real aggregate size distribution, as it replicates the soil re-wetting events that take place after rain or irrigation (Cambardella and Elliott, 1993). However, this methodology changes the chemical (pH, nutrient removal; Sainju, 2006; Seech and Beauchamp, 1988) and microbial characteristics (Nishio and Furusaka, 1970) of the isolated aggregates, which affect microbial measurements, and may result in misleading associations between aggregates and processes. In this study we addressed the co-variation between soil aggregate size classes and soil microbial processes measured in bulk soil, without affecting soil microbial communities through extraction protocols. This allows us to evaluate whether changes in soil microbial activity are linked to changes in soil structure, although we cannot directly attribute microbial processes to specific soil aggregate sizes.
COIA results indicate strong co-variation between the respiration potential, the quantity of megaaggregates and their TOC and TN contents. These associations suggest that higher availability of C and N to microorganisms would lead to increased microbial respiration, promoting aggregate formation. Bigger aggregate sizes would thus form around fresh plant material containing labile nutrients for microbial activity and mineralization (Tang et al., 2011). Recent studies showing that microbial processing of C substrates initiates the formation of aggregates >250 µm (Rabbi et al., 2020) support this explanation. Therefore, the factors affecting microbial activity (e.g. soil pH, exchangeable cations, substrate quality,

plant-microbe interactions, and environmental variables) would indirectly influence the formation of aggregates by altering the assimilation and allocation of plant-derived organic matter into different microbial products. This is in accordance with recent hypothesis indicating that metabolism and substrate use efficiency play central roles in the formation of organo-mineral associations involved in soil organic matter stabilization (Cotrufo et al., 2013; Liang et al., 2017).

The positive relationship between net N mineralization with the amount of free microaggregates and with the TN content of <2000 µm aggregates (macroaggregates, microaggregates, and silt+clay fraction) suggests that this microbial process may potentially depend on N contained in the <250 µm aggregate sizes and contribute to their formation. N Mineralization is a microbial process mediated by enzymes, which are released to the soil environment and consequently could contribute to aggregate formation. For example, the β-glucosaminidase and arylamidase enzymes (involved in N mineralization) produce amino sugars (Ekenler and Tabatabai, 2007) that may accumulate in macroaggregates and enhance the formation and stabilization of occluded microaggregates (Simpson et al., 2004) by promoting microbial activity. Favorable conditions for N mineralization in macroaggregates could also contribute to the production of organic binding agents that contribute to aggregates formation, as Muruganandam et al. (2002) found higher potential activity of enzymes associated to N mineralization in 250–1000 µm aggregates. Although no single aggregate size is responsible for N mineralization, the association of TN content of the <2000 µm aggregates with net N mineralization suggests that they may contain a larger proportion of readily organic N than bigger aggregates (Cai et al., 2016), and that differences in the aggregates microenvironment (e.g. pH, air and water availability) could influence net N mineralization.

3.5 Conclusions

Our results indicate that plantation of eucalyptus improved soil structure and sequestration of C and N in the silt+clay fraction. The cultivation of potato lead to aggregate breakdown and an increase in the amount of aggregates <2000 µm (macroaggregates, microaggregates, and silt+clay). Evaluations of alternative management practices aiming the maintenance of soil structure of cultivated fields are required to reduce the risk of erosion, and to sustain long term agricultural production. Fallowing did not lead to changes in soil structure compared to potato cultivation. The length of fallow practice (2–6 y) was too short to produce significant changes in soil structure. Soil microbial biomass (MBC) explained most of the variation in aggregate stability and size distribution compared to other C fractions. The percentage explained by oxy-hydroxides was close to that of MBC, and including their interaction, they explained 58% of the variation of aggregate stability and size distribution. Changes in MBC could indirectly influence the formation of aggregates and be indicative of early changes of soil structure. Soil respiration potential was linked to increased megaaggregate contents and increased aggregate stability, while the net N mineralization was linked to TN aggregates <250 µm (microaggregates, and silt+clay).

Acknowledgements

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Author contribution

Alejandro Coca Salazar: Conceptualization; investigation; formal analysis; writing – original draft, review and editing. Jean-Thomas Cornelis: Conceptualization; writing – review and editing, funding acquisition; supervision. Monique Carnol: Conceptualization; writing – original draft; writing – review and editing; funding acquisition; supervision.

Chapter 4

Response of ammonia-oxidizing bacteria and archaea abundance and activity to land-use changes in agricultural systems of the Central Andes

Adapted from Coca-Salazar A., Richaume A., Florio A., Carnol M. 2021. Response of ammonia-oxidizing bacteria and archaea abundance and activity to land-use changes in agricultural systems of the Central Andes. 102: 1-8. European Journal of Soil Biology. DOI: 10.1016/j.ejsobi.2020.103263

Abstract

Ammonia-oxidation is the first rate-limiting step of the nitrification process performed by ammonia-oxidizing bacteria (AOB) and archaea (AOA). The response of ammonia oxidizers to agricultural management determines the forms of nitrogen available for plant nutrition and the potential for nitrate leaching, nitrous oxide emissions, and soil acidification. We investigated the potential nitrification rates (PNR) of AOA and AOB through the use of a specific inhibitor of bacterial nitrification, and the *amoA* gene abundance of AOB and AOA under potato, fallow and eucalyptus land uses in an agricultural system in the Central Andes of Bolivia. AOA dominated PNR and *amoA* gene abundance under all land uses. The ratio of AOA to AOB abundance decreased with soil pH, due to higher AOB abundances under the less acid soils of potato crops. Eucalyptus led to reduced AOB *amoA* abundances and PNR of both AOA and AOB, while PNR were highest under potato soils, and the contribution of AOB to total PNR increased. Specific PNR, as expressed per *amoA* gene copy numbers, was 12, 14 and 62 times higher for AOB than for AOA in potato, fallow and eucalyptus soils, respectively. AOB and AOA PNR were positively related to their respective *amoA* gene copy numbers, but for AOA the relationship depended on land use. This study demonstrates the interest for measuring separately nitrification rates of AOA and AOB for a mechanistic understanding of nitrification in different environments, as well as the importance of measuring process rates for assessing the environmental consequences of land use management.

Keywords: Nitrification, Ammonia-oxidizing bacteria and archaea, soil pH, *Solanum tuberosum*, *Eucalyptus globulus*, Bolivia

4.1 Introduction

Nitrification is the biological oxidation of ammonia to nitrate followed by the oxidation of nitrite to nitrate. Ammonia oxidation is carried out by ammonia-oxidizing archaea (AOA) and bacteria (AOB), while the recently discovered comammox bacteria are capable of performing both steps (Kits et al., 2017; Stein, 2019). When AOB and AOA are involved, ammonia oxidation is the rate limiting process, and their relative contribution to nitrate production rates in soils is still under debate. Niche differentiation of ammonia oxidizers was initially attributed to differences in ammonia affinities, with AOB showing lower affinity and requiring higher substrate availability (Jung et al., 2011; Martens-Habbena et al., 2009; Park et al., 2010). For example, Martens-Habbena et al. (2009) demonstrated that AOA are capable of oxidizing ammonia and to replicate at ammonium concentration below 10 nM, which is 100 times lower than the minimum concentration required for AOB activity and replication (Bollmann et al., 2002). AOA were thus considered to be more relevant in oligotrophic environments, but recent research on their metabolic characteristics revealed similar affinities between AOA and AOB in soils (Kits et al., 2017; Zhang et al., 2020). Further, pH strongly influences ammonia-oxidizing prokaryotes through controls on free ammonia, and lower ammonia availability under acid conditions (Gonzales-Cabaleiro et al., 2019; Suzuki et al., 1974) would favor archaea over bacteria. Also specific metabolic adaptations, such as the vacuole-type ATPases, may explain growth of *Thaumarchaeota* in low pH environments (Wang et al., 2019).

Concomitantly, increased relative abundances of AOB and AOA in environmental samples through the measurement of the *amoA* gene (which encodes for the alpha-subunit of the ammonia-monooxygenase enzyme) indicated AOA dominance over AOB in numerous environments (Clark et al., 2020; Li et al., 2012; Sterngren et al., 2015), and specifically under acidic conditions (He et al., 2007; 2012; Leininger et al., 2006; Lu et al., 2015; Qin et al., 2013). However, while in some acidic environments potential nitrification rates (PNR, assessing the activity of autotroph nitrifiers), show strong positive associations with AOA *amoA* gene copies (Lu et al., 2015), other studies report weak or no correlation (Gao et al., 2018; Yao et al., 2011; Zhang et al., 2016). These discrepancies may be attributed to higher specific oxidation rates of AOB, as shown in cell cultures (Jiang and Bakken, 1999; Jung et al., 2011; Park et al., 2010), and evidence that at some sites acid-tolerant AOB may significantly contribute to nitrification (Carnol et al., 2002; Hayatsu et al., 2017). Given that studies linking ammonia-oxidizer activity and abundance in environmental soil samples rarely differentiate between the contribution of AOA and AOB to total PNR, it has been difficult to interpret the ecological relevance of AOA or AOB *amoA* gene abundances, and moreover, it is not known whether the higher specific oxidation rates of AOB compared to AOA determined under laboratory conditions are also relevant in the environment. Evaluation of the relative contribution of archaea and bacteria to PNR provides evidence of their ecological relevance and the potential consequences of the changes in the AOA and AOB abundances.

The contribution of AOA and AOB to nitrification can be evaluated by the use of specific inhibitors, such as 1-octyne and allylthiourea. Allylthiourea (ATU) is a metabolic inhibitor that selectively chelates the copper in the active site of the ammonia mono-oxygenase enzyme and inhibits AOB activity (Ginestet et al., 1998; Jung et al., 2011; Taylor et al., 2010). ATU concentrations above 80 µM have been demonstrated to inhibit bacterial ammonia oxidation, while some AOA are not affected, even at concentrations of 500 µM (Ginestet et al., 1998; Jung et al., 2011; Martens-Habbena et al., 2015). ATU has been successfully used in kinetics studies of nitrification (Jung et al., 2011; Munz et al., 2010) and studies of waste water treatment (Adamczyk et al., 2003; Rattier et al., 2014; Zhou and Oleszkiewicz, 2010). Its incorporation in PNR measurements can thus be used to differentiate the nitrification performed by AOB and AOA, and contribute to the understanding of their respective role in the environment.

Changes in soil chemistry, especially pH, caused by land use can also differentially affect the nitrification process and the abundance and activity of AOB and AOA. For example, a study across different land uses under the same soil type reported changes in nitrification and *amoA* abundance following a pH change through acidification or liming (Zhao et al., 2020). Similarly, several studies of agricultural land uses reported that soil pH is the main variable explaining changes in the ammonia oxidizers community and potential nitrification rates under different fertilization regimes but with a different effect on AOA and AOB (Nicol et al., 2008; Sun et al., 2019; Wessén et al., 2010). However, while the central role of soil pH on determining niche differentiation has been recognized, the relative contribution of AOB and AOA to nitrification under different land uses remains poorly understood. Evaluation of AOB and AOA potential nitrification and abundances is essential to understand their role in the environment and the potential consequences of land-use change on the N cycle, particularly in agricultural systems where their activity determines the type of mineral nitrogen available for plants, but also contributes to nitrate leaching and ground water contamination (Subbarao et al., 2012), nitric and nitrous oxide gas emissions (Kampschreur et al., 2010), and soil acidification (Bolan et al., 1991; Zhao et al., 2020).

We studied AOB and AOA PNR in relation to *amoA* gene abundance in agricultural areas of the mountainous Andean range of Bolivia characterized by acidic soil pH (Condori et al., 1997). These Andean landscapes are dominated by three types of land use: (1) intensively cultivated fields in which potato constitutes the main crop planted during 2-4 consecutive growing seasons, followed by secondary crops (*Vicia faba* and *Ordeum vulgare*) after which the cycle is repeated again or fields are left fallow; (2) agricultural fields that are left fallow for a maximum of 6 years and are colonized by self-regenerated herbaceous vegetation, aiming at fertility restoration; (3) introduced *Eucalyptus globulus* L. plantations that replaced agricultural areas as an alternative economic income to farmers.

The objective of this study was to determine the relationship between AOA and AOB abundances and total, AOA and AOB nitrification rates in response to the three land uses. Given the acidity of the soils we hypothesized a dominance of AOA activity and abundance under fallow fields and eucalyptus plantations, but due to organic and mineral fertilization

of cultivated fields, we expect higher activity and abundance of AOB in the potato cultivated fields.

4.2 Materials and methods

4.2.1 Study site and soil sampling

The study was conducted in the fields of the Chullchunqani Community (17°32'30" - 17°33'30" S, 065°20'08" - 065°21'36" W, altitude range 3100-3400 m), an agricultural community located in the Eastern branch of the Andes range, characterized by a rainy summer season (November-March) and a dry winter season (April-October) (Navarro and Maldonado, 2002; Pestalozzi, 2000), with a mean annual rainfall of 500.7 mm, and a mean annual temperature of 17.9 °C (SENAMHI, 2016). The topography is characterized by secondary mountain ranges of the Andean region, with soils classified as Cambisols (Ministerio de Medio Ambiente y Agua, 2014).

The potato crops, fallow fields and eucalyptus plantations are interspersed within the landscape in areas of ca. 0.5 - 1 ha, defined here as "plots". Potato fields are tilled (ca. 20 cm depth) for soil preparation, and fertilizers, mainly chicken manure are added when sowing. Additional fertilization and irrigation is applied in varying amounts according to farmers' personal judgement, and harvesting is conducted manually. Before conversion to fallow, remaining aboveground biomass is incorporated by tillage and the fields are then left unmanaged. Fields converted to eucalyptus plantations are not managed after seedling plantation. Eight plots of each land-use type were selected within an area of ca. 4 km^2 based on the following criteria: agricultural plots in which potato had been grown during the last rainy season, fallow plots (2-6 years old) in which the spontaneously grown vegetation (grass-shrubland of semiarid high Andes; (Navarro and Maldonado, 2002)) fully covered the soil, and Eucalyptus (*E. globulus)* plantations (minimum 5 years since plantation).

Soils from potato, fallow and eucalyptus land uses were sampled in February 2017, at the end of the rainy season. Within each plot, three randomly selected sampling points were established at a minimum distance of 10 m. At each sampling point, one composite soil sample was taken with a shovel (one central sample and four individual samples taken two meters from this central sample; 20 cm depth). The thin organic layer (<0.5 cm) of the eucalyptus plots was discarded. Samples were homogenized, sieved (2 mm mesh) under sterile conditions and stored at 4 °C for potential nitrification measurements. From one randomly selected soil sample per plot, a sub-sample was freeze-dried and stored for molecular analyses.

4.2.2 Soil pH and potential nitrification

Soil pH_{KCl} and potential nitrification rates (PNR) were determined on all three soil samples taken in each plot. Soil pH was determined in KCl 1M (1:2 w/v) (HI2550, HANNA instruments) (Allen, 1989). PNR was determined using the shaken soil slurry method (Hart, et al., 1994), with and without allylthiourea (80 µM), a selective inhibitor of bacterial

nitrification (Taylor et al., 2010). Briefly, fifteen grams of soil were shaken at 180 rpm in 100 ml nutrient solution (pH 7.2, 1 mM PO_4^{3-}, 1.5 mM NH_4^+) at 20°C in the dark. After 2, 5, 23 and 26 h of incubation, 15 ml of homogenized soil slurry was sampled, centrifuged, filtered (Whatmann 595 ½) and stored at -20 °C until analyses. Nitrate content of the extracts was analyzed colorimetrically with a continuous flow autoanalyzer (BranLuebbe, SPX Process Equipment, Germany). PNR were calculated by linear regression of NO_3^--N concentrations over time. We were thus able to determine total PNR and AOA-PNR (allylthiourea treated soil-slurry). The AOB-PNR were calculated as the difference between total PNR and AOA-PNR. PNR assesses the maximum nitrification rate of the nitrifier population present in a soil sample at the time of sampling. The short incubation time, high NH_4^+ concentrations and oxygenation assure the absence of nitrifier's population growth, denitrification and N immobilization during the incubation, so that net rates are equivalent to gross rates (Hart, et al., 1994). Further, the buffer solution is dilute enough so that the final pH of the soil slurry is close to the soil-water solution (Hart, et al., 1994). The high ammonium supply might influence the activity of some species (both AOA and AOB), but not lead to a selective advantage of one group, as recent studies revealed a similar range of ammonia affinities between AOA and AOB from soils (Kits et al., 2017; Zhang et al., 2020).

4.2.3 amoA gene abundance

DNA was extracted from 0.25 g freeze-dried soil samples using the DNeasy PowerSoil Pro kit (QIAGEN) according the manufacturer's protocol on one randomly selected sample per plot. The DNA concentration and quality were measured using a Qubit™ fluorometer (Invitrogen). Archaeal and bacterial nitrifier abundances were determined by quantitative PCR targeting the *amoA* gene sequences of each domain. The gene primers amoA_1F and amoA_2R were used for bacteria (Rotthauwe et al., 1997), and the primers CrenamoA23f and CrenamoA616r for archaea (Tourna et al., 2008). A reaction mix containing 0.5 µM of each bacterial *amoA* primer or 0.75 µM of CrenamoA616r and 1 µM of CrenamoA23f primers, 2% bovine serum albumin (BSA), 1X of QuantiTect SybrGreen PCR Master Mix (Qiagen, Courtaboeuf, France) and 10 ng of soil DNA where run in duplicate on Lightcycler 480 (Roche Diagnostics, Meylan, France). The qPCR efficiency for the bacteria *amoA* was 88.8%, and for the archaea *amoA* was 79.2%. For Archaea, the highest qPCR efficiency and the best melting curve quality with the LightCycler (Roche Molecular Biochemicals) were obtained using different concentrations for forward and reverse primers. Such asymmetric amplification had been shown to improve the qPCR efficiency and melting curve quality using the LightCycler (Barratt and Mackay, 2002). Run details are given in Simonin et al. (Simonin et al., 2016). We calculated the *amoA* gene abundance for AOA (AOA *amoA*) and AOB (AOB *amoA*), and the *amoA*-AOA:*amoA*-AOB ratio. PNR per unit *amoA* gene copies for AOA and AOB were calculated by dividing their PNR by their respective number of *amoA* copies.

4.2.4 Statistical analyses

Differences in soil pH_{KCl} and PNR between land uses were assessed by linear mixed effects models (data for three samples per plot) for AOA and AOB, followed by post hoc Tukey test for linear mixed models (Faria et al., 2018; Mangiafico, 2015). The models included "plot" as random factor to account for the non-independence of the three soil samples taken in each plot (Mangiafico, 2015).

Differences in *amoA* gene copy numbers, *amoA*-AOA: *amoA*-AOB, and PNR per unit *amoA* for AOB and AOA between land uses were tested with one-way ANOVA and post hoc Tukey tests (data from one sample per plot). The data of *amoA* gene copy numbers and *amoA*-AOA: *amoA*-AOB were log-transformed to meet normality and homoscedasticity assumptions.

We assessed the relationships of PNR with soil pH_{KCl}, AOA *amoA* and AOB *amoA* by fitting linear mixed effects models that allowed for random intercepts and slopes (Zuur et al., 2009). The marginal r-square $R^2_{LMM(m)}$ (variance explained by fixed effects of the model) were calculated according to Jaeger *et al*. (Jaeger et al., 2016), and separate models for each land use were used to calculate individual regression coefficients (R^2). To determine if slopes differed between land uses, we compared the models fitted with and without random slopes. A model AIC lower by 2 units is considered to represent a better fit. The relationship of *amoA*-AOA:*amoA*-AOB with soil pH_{KCl} was assessed by fitting a simple non-linear model.

All statistical analyses were conducted with R software (R Core Team, 2018) using the packages car (Fox and Weisger, 2011), nlme (Pinheiro et al., 2018), nltoosl (Baty et al., 2015), r2glmm (Jaeger, 2017), and TukeyC (Faria et al., 2018).

4.3 Results

4.3.1 Effects of land use on soil pH and potential nitrification rates

Average soil pH_{KCl} was 4.3 for soils in potato and fallow plots, and 4.0 for soils under eucalyptus without significant differences between land uses (Table 4.1). Total PNR ranged from 0.00 to 0.51 mg NO_3^--N kg^{-1} d^{-1} with significantly higher rates under potato and lower rates under eucalyptus plantations. The AOB-PNR ranged from 0.00 to 0.24 mg NO_3^--N kg^{-1} d^{-1} with significantly higher values in potato crops compared to eucalyptus and fallow, whereas AOA-PNR ranged from 0.01 to 0.35 mg NO_3^--N kg^{-1} d^{-1} with significantly higher rates in potato crops and in fallow fields. Archaeal contribution to total nitrification rates ranged from 39 to 100% and, on average, AOA accounted for 64, 82 and 96% of total nitrification in potato, fallow and eucalyptus soils, respectively (S1 of Supplementary material).

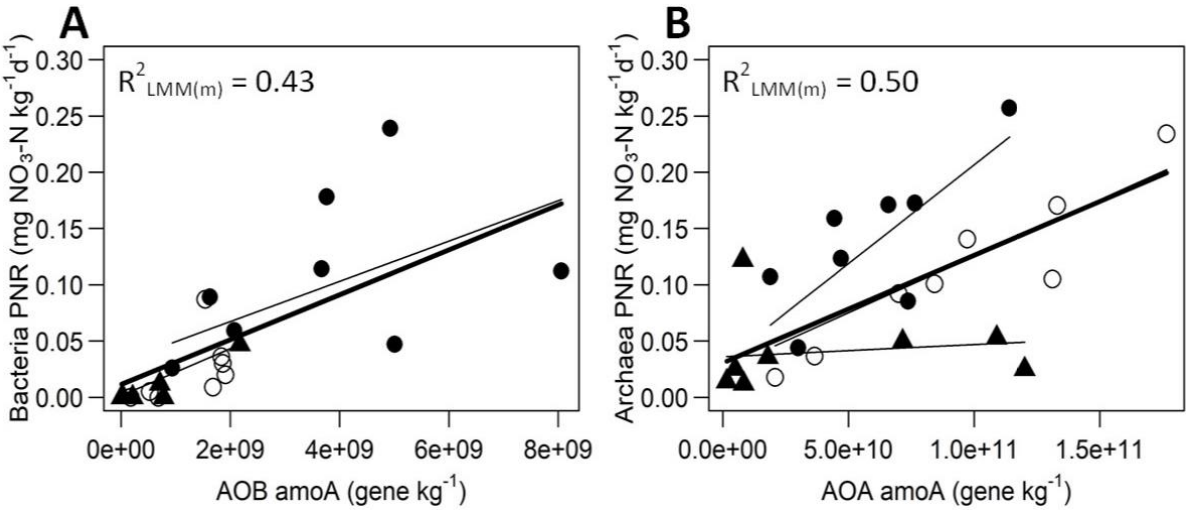

Figure 4.1 Relationships of AOB and AOA amoA gene copy numbers with their PNR (A and B, respectively). The thick lines were fitted for all data points (n=24) and thin lines were fitted for each land use separately (n=8), points represent soils under potato (●), fallow (○) and eucalyptus (▲) land use. In A the fitted lines of potato and fallow overlap and in B the fitted line for fallow overlaps with the general regression line.

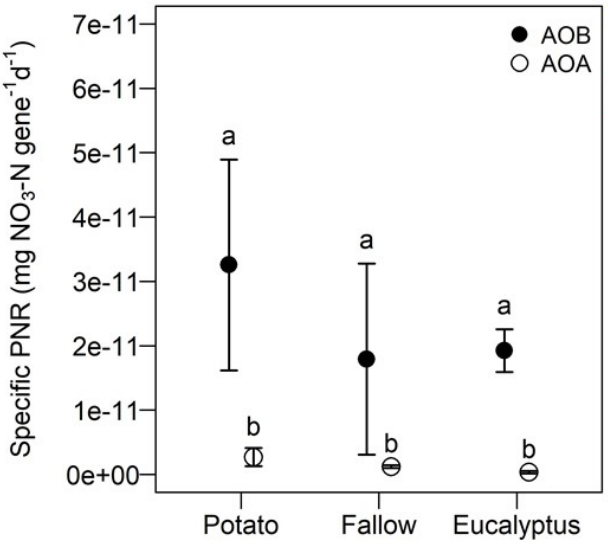

Figure 4.2 Specific potential nitrification rates per land use. AOA and AOB specific potential nitrification rates (potential nitrification per unit of amoA gene copies) were no significantly different between land uses (p-value >0.05, ANOVA and Tukey tests). Letters indicate significant differences between AOA and AOB for each land use (p-value >0.05, Tukey tests).

4.3.2 Abundance of AOA and AOB amoA gene copies under the three land uses

The AOB *amoA* abundance ranged from 1.1×10^7 to 8.1×10^9 gene copies per kg of dry soil with significantly lower values under eucalyptus plantations compared to potato and fallow soils (Table 4.1). AOA *amoA* abundance ranged from 1.5×10^9 to 1.8×10^{11} gene copies per kg of dry soil without significant differences between land uses. The proportion of AOA *amoA* to the total *amoA* gene copies ranged between 86 to 100%, and on average accounted for 93% of total *amoA* copies under potato and for 98% under fallow and eucalyptus land

uses (S1 of Supplementary material). Except for one extreme value under eucalyptus (6777), *amoA*-AOA:*amoA*-AOB ratios ranged from 6 to 468. The mean *amoA*-AOA:*amoA*-AOB ratios were significantly higher under eucalyptus plantations, compared to potato land use (Table 4.1).

4.3.3 Relationships between the nitrification potential, amoA gene abundance and pH

Total PNR showed a positive relationship with both AOB *amoA* ($R^2_{LMM(m)}$=0.39) and AOA *amoA* ($R^2_{LMM(m)}$=0.61) gene abundances at landscape level (S2 of Supplementary material). At land use level total PNR was positively correlated with AOA *amoA* under potato and fallow (R^2=0.64 and 0.69, respectively), and with AOB *amoA* under eucalyptus (R^2=0.94, S3 of supplementary material). AOB-PNR and AOA-PNR had positive relationships with their respective *amoA* gene copy numbers, with marginal r-squares $R^2_{LMM(m)}$ of 0.50 for AOA, and 0.43 for AOB (Figure 4.1). For AOA, the random slope model performed better than the fixed slope model (Δ_{AIC}=3.7, *p*-value=0.02), but not for AOB (Δ_{AIC}=4, *P*-value=0.98). The individual relationships between AOA-PNR and AOA *amoA* within each land use were positive, with regression coefficients of 0.50, 0.86, 0.23 for potato, fallow and eucalyptus soils, respectively (S4 of supplementary material). The relationships of AOB-PNR and AOB *amoA* in potato soils was not significant (R^2=0.01), it was weak in fallow soils (R^2=0.14) and strong under eucalyptus plantations (R^2=0.86, S4 of supplementary material).

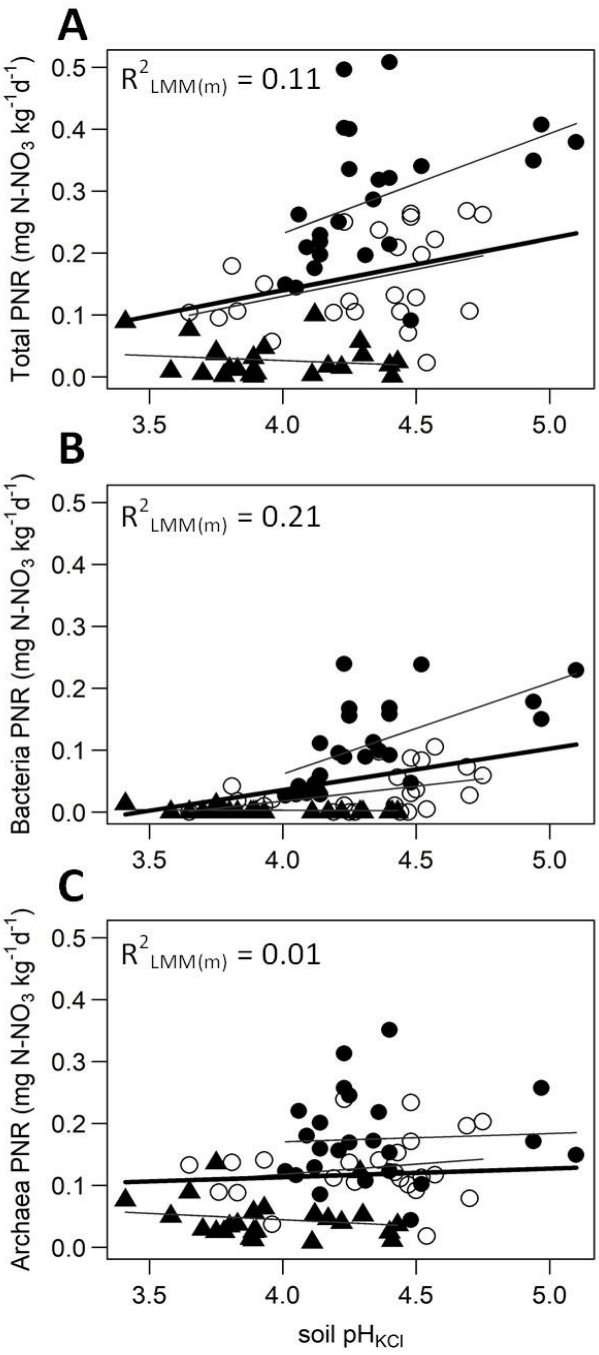

Figure 4.3 Relationship of potential nitrification rates (PNR) and soil pH_{KCl}. Relationship of total (A), bacteria (B) and archaea (C) PNR with soil pH_{KCl}. The thick lines were fitted for all data points (n=72, three samples per plot) and thin lines were fitted for all data points (n=72, three samples per plot) and thin lines were fitted for each land use (n=24), points represent soils under potato (●), fallow (○) and eucalyptus (▲) land uses.

Table 4.1 Soil pH_{KCl}, potential nitrification rates (PNR), amoA gene copy numbers and amoA-AOA:amoA-AOB ratio under three land uses (mean ± standard deviation; n=8). Different letters indicate significant differences between land uses (p-value <0.05, nested ANOVA and Tukey tests). Values were calculated using the average values of the three samples taken in each plot.

	Soil pH_{KCl}	Total PNR (mg NO_3^--N kg^{-1} d^{-1})	AOB-PNR (mg NO_3^--N kg^{-1} d^{-1})	AOA-PNR (mg NO_3^--N kg^{-1} d^{-1})	AOB *amoA* (gene kg^{-1})	AOA *amoA* (gene kg^{-1})	*amoA*-AOA:*amoA*-AOB ratio
Potato	4.3±0.3 a	0.29±0.11 a	0.11±0.07 a	0.18±0.07 a	$3.7\times10^{9}\pm2.3\times10^{9}$ a	$5.9\times10^{10}\pm3.0\times10^{10}$ a	19.94±13.61 b
Fallow	4.3±0.3 a	0.16±0.07 b	0.03±0.03 b	0.13±0.05 a	$1.3\times10^{9}\pm7.0\times10^{8}$ a	$9.4\times10^{10}\pm5.2\times10^{10}$ a	124.46±148.88 ab
Eucalyptus	4.0±0.3 a	0.03±0.03 c	0.00±0.01 b	0.04±0.03 b	$4.9\times10^{8}\pm7.5\times10^{8}$ b	$4.3\times10^{10}\pm4.9\times10^{9}$ a	140.71±118.21 a

Specific PNR (AOA and AOB PNR per unit of their respective *amoA* gene copies) indicated no significant differences between land uses (Figure 4.2), and significantly higher specific PNR for AOB (t-test, data not shown). On average, the specific PNR of AOB was 12, 14 and 62 times higher than the specific PNR of AOA under potato, fallow and eucalyptus soils, respectively.

Total PNR and AOB-PNR showed weak positive relationships with soil pH_{KCl} ($R^2_{LMM(m)}$=0.11 and 0.21, respectively) with statistical significant slopes (*p*-value <0.01), while there was no significant relationship (*P*-value of slope=0.54) between AOA-PNR and PH_{KCl} ($R^2_{LMM(m)}$=0.01). Regression coefficients of total PNR and soil pH_{KCl} were 0.14, 0.10 and 0.00 for potato, fallow and eucalyptus soils, and the regression coefficients of AOB-PNR with soil pH_{KCl} were 0.37, 0.16, 0.00 under potato, fallow and eucalyptus soils, respectively (Figure 4.3, S5 of supplementary material). The *amoA*-AOA:*amoA*-AOB ratio and soil pH_{KCl} showed a negative power-convex relationship (Figure 4.4).

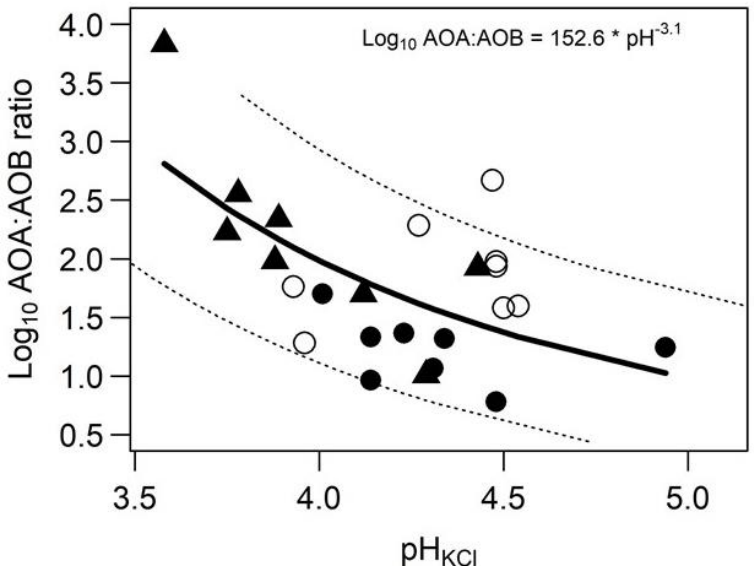

Figure 4.4 Archaea-to-bacteria amoA ratio and soil pH_{KCl}. Relationship between the amoA-AOA: amoA-AOB ratio and soil pH_{KCl} for potato soils (●), fallow soils (○) and eucalyptus plantations (▲). The thick line represents the fitted curve and the dashed lines are confidence intervals (n=24).

4.4 Discussion

4.4.1 AOB and AOA activity and abundance in response to land use

This study presents the first report addressing the consequences of land use on the activity and abundance of ammonia-oxidizer bacteria and archaea in agricultural ecosystems in the Central Andean region. Total PNR under potato and fallow were relatively low (0.00-0.51 mg NO_3^--N kg^{-1} d^{-1}, Table 4.1) compared to potential nitrification measured in fertilized agricultural soils (2.2-105 mg NO_3^--N kg^{-1} d^{-1}; Chu et al., 2007; Zhang & Ji, 2018), grassland soils (1.1-3.4 mg NO_3^--N kg^{-1} d^{-1}; Veresoglou et al., 2011), but total PNR under eucalyptus (Table 4.1) were similar to previous reports for eucalyptus plantations (0.01-0.03 mg NO_3^--N kg^{-1} d^{-1}; Zhang et al., 2016). Nitrification was largely attributed to AOA, responsible for 64% to 96% of total potential nitrification (S1 of Supplementary material). Under eucalyptus AOA accounted for 96% of total PNR but both rates for AOB and AOA were low compared to the other land uses (Table 4.1), which can be explained by the inhibitory effect of organic compounds (i.e. terpenes and caffeic acid) produced by this tree on bacterial and archaeal nitrification (Sauder et al., 2016; White, 1986). Potential nitrification under potato was dominated by AOA but the relative contribution of AOB was 36% of total PNR despite they accounted for only 7% of total *amoA* copies (S1 of Supplementary material). The higher relative contribution of AOB to potential nitrification could be explained by the competitive advantage of AOB under higher ammonium concentrations due to inorganic N fertilization (Chu et al., 2008; French et al., 2012). Also, chicken manure additions may have contributed to increased AOB activity by promoting the formation of soil aggregates with favorable micro environmental conditions (neutral pH and high ammonia concentration; (Burton and Prosser, 2001; de Boer et al., 1991). Moreover, the significant increase in AOB activity could be related to the higher ammonia oxidation rate per AOB cell (4-23 fmol NO_2^- $cell^{-1}$ h^{-1}; Prosser & Nicol, 2012) compared to AOA (0.07-2.5 fmol NO_2^- $cell^{-1}$ h^{-1}; Lehtovirta-Morley et al., 2016), which is reflected in the higher specific PNR (PNR per *amoA* gene copies) of AOB. Although *amoA* gene copy numbers do not strictly reflect cell numbers, as AOB and AOA vary in the number of *amoA* copies in their genetic material (Klotz & Norton, 1995; Norton et al., 1996; Pedneault et al., 2014; Wang & Chen, 2018), the specific PNR indicate that AOB contribution to nitrification per unit of *amoA* copy is 12, 14 and 62 times higher than AOA under potato, fallow and eucalyptus, respectively (Figure 4.2). Upon conversion to fallow, AOB-PNR decreased and AOA contribution to total nitrification increased to 82% (S1 of Supplementary material). This could be due to the absence of N fertilization and the dependency on N supplied by mineralization of organic matter entering the soil from colonizing vegetation.

Results revealed that AOA were also dominant in terms of gene abundance with the AOA *amoA* copy numbers one to two orders of magnitude higher than AOB *amoA* (Table 4.1). The *amoA*-AOA*:amoA*-AOB ratios were thus situated between 20 and 141 (Table 4.1), and the differences between land uses resulted from changes of AOB *amoA* abundance, as the AOA *amoA* remained unchanged (Table 4.1). Despite soil pH did not differ between land uses, a wider pH range was observed under potato, and changes in the dominance ratio are

likely driven by soil pH because minor variations have strong effects on AOB abundance (Shen et al., 2008). Increasing acidic conditions would thus lead to stronger AOA dominance as observed under eucalyptus, which had the highest *amoA*-AOA:*amoA*-AOB ratios and reported one extreme value (6777) that corresponded to the soil sample with the lowest pH (3.5). Yao et al. (2011) reported a similar relationship between *amoA*-AOA:*amoA*-AOB and soil pH, in accordance with studies indicating that pH is the major factor determining AOB and AOA niche differentiation (Nicol et al., 2008; Sun et al., 2019). Soil pH showed a significant positive relationship with total PNR (R^2=0.14, S5 of supplementary material) and with AOB-PNR under potato and fallow (R^2=0.37 and R^2=0.16 respectively, S5 of Supplementary material). These results suggest that under higher pH, nitrification rates would be mostly controlled by AOB, likely due to increased ammonia NH_3 availability. This is in accordance with previous studies indicating reduced AOB activity with increasing soil acidity (French et al., 2012; Zhang et al., 2012). In contrast, under low pH, AOA would be most important, but the control of nitrification rates would also depend on other factors than pH or AOA gene copy number, as illustrated by the absence of correlation between pH and AOA-PNR (Figure 4.3C, S5 of supplementary material), and varying slopes between AOA *amoA* and AOA-PNR under the three land uses (Figure 4.1B). Ammonia-oxidizer community composition and diversity (Qin et al., 2013) could be important in controlling AOA activity within the acidic range of the studied soils.

4.4.2 Relationship of potential nitrification rates with amoA abundance

Since the discovery of AOA, numerous studies have documented their dominance in different environments through the measurement of their *amoA* gene copy numbers (Clark et al., 2020; Leininger et al., 2006; Li et al., 2012; Lu et al., 2015; Qin et al., 2013; Sterngren et al., 2015), and particularly, in agricultural systems where AOA and AOB activities determine the N fertilization efficiency and potential N loss through nitrate leaching (Norton & Ouyang, 2019; G. V. Subbarao et al., 2009). These studies have raised the question whether this dominance reflects their functional importance in nitrification, but few have included nitrification rate measurements. Studies assessing *amoA* gene abundance and total PNR usually have associated the presence of correlations as indicative of the functional importance of AOB or AOA in the environment (Gao et al., 2018; Qin et al., 2013; Yao et al., 2011; Zhang et al., 2016). However, our results show that these relationships do not reflect the real contribution of AOA and AOB ammonia oxidizers to nitrification, as well as previous studies reporting negative correlations (Gao et al., 2018). Indeed, in our study, total PNR was strongly positively correlated with AOB *amoA*, despite that rate measurements showed that nitrification was mainly due to AOA (Table 4.1, S1 of Supplementary material). AOB and AOA relevance to nitrification process rates should thus be determined by separately measuring their nitrification rates and not be inferred from relationships of *amoA* with total PNR.

Increases in the AOB *amoA* and AOA *amoA* abundances were associated with their respective PNR (Figure 4.1), and consequently with ammonia-oxidizers community size (Rahalkar et al., 2009) but the relationships differed for AOB and AOA. The AOB *amoA*

and AOB-PNR relationship was strong at landscape level, but relationships were weak within land uses and had similar low slopes (S4 of supplementary material), likely due to the low nitrification rates and narrow *amoA* abundance range. These results suggest that soil management practices known to increase the AOB *amoA* abundance (e.g. fertilization, liming) could be expected to increase AOB nitrification (Bossolani et al., 2020; Shen et al., 2008). The AOA *amoA* and AOA-PNR relationships differed between land uses, with strong positive relationships and greater slopes under potato and fallow soils, but no significant relation under eucalyptus plantations (S4 of supplementary material). Changes in the environmental factors under eucalyptus may contribute to explain lower AOA activity than expected by the AOA *amoA* abundance alone. For example, the presence of AOA nitrification inhibitors produced by this tree (Sauder et al., 2016) may have contributed to the low AOA-PNR despite the presence of AOA. Also, changes in the organic carbon content, which have been reported to affect AOA activity by increasing competition for available ammonia and oxygen with heterotrophs (Liu et al., 2018; Sun et al., 2019; Wessén et al., 2010) could explain low PNR under eucalyptus. Consequently, the relationships between AOA abundance and activity at low pH is land-use dependent, and changes in AOA activity cannot be inferred from changes in the AOA *amoA* abundance alone.

4.5 Conclusions

We demonstrated that AOA dominated in terms of abundance and activity under the three land uses studied. Eucalyptus led to decreased AOB abundance, and AOA and AOB nitrification rates, while under potato nitrification rates were highest and the relative contribution of AOB increased. The AOA-to-AOB *amoA* ratio decreased with pH, and was driven by the increase of AOB *amoA* with decreasing soil acidity. The relation of *amoA* gene copy numbers and nitrification rates depended on land use, indicating that gene abundance measurements alone are not sufficient for assessing AOB and AOA activity. Therefore, rate measurements of AOB and AOA are essential to determine the environmental consequences of land-use management. Our results indicate that in these agricultural soils, the role of AOB might become predominant in controlling nitrification rates with increasing pH and N inputs, while under low pH AOA would be most important with process rates controlled by other factors than pH and gene abundances.

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Author contributions

Alejandro Coca-Salazar and Monique Carnol designed the study and wrote the manuscript. Material preparation, data collection and analysis were performed by Alejandro Coca-Salazar. Agnes Richaume and Alessandro Florio performed qPCR analyses. All authors commented, read and approved the final manuscript.

Chapter 5

Effects of chicken manure management on soil quality and plant development

Adapted from Coca-Salazar A. and Carnol M. Effects chicken manure management on soil quality and plant development (manuscript)

Abstract

Chicken manure waste generation and its use as fertilizer for agricultural production have increased worldwide. The high demands for organic fertilizers and the costs of proper manure management (composting) have led to the frequent application of raw or incompletely composted chicken manure to agricultural fields worldwide, causing potential negative consequences for air, soil and water quality. In the agricultural highlands of the Andes, Bolivia, chicken manure at varying stages of composting is now applied yearly. For formulating management advice, it becomes essential to assess the potential consequences of such practices for crops and soils. We evaluated the effects of compost pile turning frequencies (no turning, every 4 weeks and every 2 weeks) on chicken compost maturity and stability during 150-days and the effects of the compost produced on soil properties, seed germination and plant growth (shoot length and plant biomass production). Faster manure stabilization was observed in piles subjected to turning events. Soil fertilization with chicken manure increased hot water extractable C and N fractions, respiration potential, pH, seed germination and growth of *Avena sativa* (shoot length and plant biomass production). The use of manure extracts at early stages of decomposition resulted in apparent root damage to *A. sativa* seedlings. This phytotoxic effect was lower in manure piles subjected to turning events after 65 days of composting. We recommend that manure piles should be composted a minimum of 100 days, conducting one turning event every four weeks to ensure proper manure stabilization and maturation before application to the field.

Keywords: Composting, soil respiration, hot water extractable carbon, microbial biomass, root damage, poultry manure.

5.1 Introduction

Over the past decades, world food production has increased steadily in order to meet the growing demand of the population (Tilman et al., 2002). Much of the increase in food production has been due to higher inputs of chemical fertilizers (Motesharezadeh et al., 2017; Tilman et al., 2002). However, long-term chemical fertilization may cause soil deterioration, greenhouse gas emissions, and water contamination (Chai et al., 2019; Savci, 2012; Tilman et al., 2001), which may affect long-term agricultural production and sustainability. An alternative to chemical fertilization is the use organic fertilizers, which are known to improve soil texture and aeration, increase organic matter content, nutrient and water retention capacity, as well as to stimulate healthy root development (Duong, 2013). Organic fertilization would thus contribute to long-term sustainable agricultural production and constitute a suitable option for utilizing animal wastes (Dróżdż et al., 2020).

The availability of organic materials, such as animal manures, and their use in agricultural production have increased worldwide (Dróżdż et al., 2020; Godfray et al., 2010; Kollnig, 2020). The effects of fully composted and fresh animal manure fertilization on soil characteristics and plant growth have been intensively studied. It is known that composted manure can improve soil structure and nutrient retention capacity by increasing soil organic matter, and ultimately enhance plant growth (Duong, 2013; Thomas et al., 2019). On the other hand, the use of fresh manures may lead to potential phytotoxic effects (Bernal et al., 2009; Britto and Kronzucker, 2002; Gajbhiye, 2013), soil contamination with pathogens, antibiotics and trace elements (Deng et al., 2020; Kyakuwaire et al., 2019; Yang et al., 2014), but may also increase crop yields compared to composted manure due to high nutrient availability (Augstburger, 1983). Nevertheless, the increasing demand of organic fertilizers, the high cost and intense labor required for composting discouraged farmers and led to applications of fresh manure all over the world (Dróżdż et al., 2020; Kyakuwaire et al., 2019; Ndambi et al., 2019; Yang et al., 2014). Commonly, recommendations of proper manure management practices before its utilization are not followed. The local consequences of this management practice are rarely addressed, but their study is necessary for proper communication to farmers and for providing management advice.

Composting is an aerobic process during which the organic matter is decomposed and complexed through microbial activity into more stable and nutrient-rich compounds than the original material. It is divided into four overlapping stages (mesophilic, thermophilic, cooling down and maturation) during which compost properties vary widely, depending on the characteristics of the raw material, as well as composting practices such as turning of manure (Bernal et al., 2009). During composting, complexation of labile organic compounds (compost stabilization) and degradation of phytotoxic organic compounds (compost maturation) take place (Khalil and Hassouna, 2013; Raut et al., 2008). Compost management practices such as turning of manure may accelerate these processes by maintaining aerobic conditions that promote microbial activity (Diaz et al., 1993), while the absence of turning events could slow down the composting process (Khalil and Hassouna, 2013). Despite extensive research conducted on the effects on fully composted manure on soil

characteristics, information on the effects of manure at different stages of decomposition on plant growth and soil characteristics is limited. Understanding the effects of manure at different stages of the composting process on soils and plant growth are however of central importance for contributing to an efficient and optimal use of organic fertilizers.

In the Andean region of Bolivia, potato production has been historically based on three to four cultivation cycles followed by long fallow periods ranging between 10 and 50 years. This was considered to be a sustainable strategy for managing fertility of soils with low nutrient contents and for achieving high potato yields (Pestalozzi, 2000; Zimmerer, 1993). However, agricultural intensification lead to progressive shortening of fallow periods to 2-6 years, and the necessity to develop alternative management practices. Soil fertilization has been adopted as an alternative for managing soil fertility in these agricultural areas. Due to the restrictive prize of industrial chemical fertilizers, organic alternatives, such as chicken manure, gained attention due to its low cost, high nutrient content and increasing availability (Augstburger, 1989, 1983; Kollnig, 2020). Currently, chicken manure is the most important fertilizer for potato production in the high mountainous areas and mesotermic valleys of the country. Despite governmental efforts to provided technical support for fertilization, soil fertilization practices rely on communal decisions or farmers' personal judgement (Eguívar and Garcia, 2006) that commonly lead to excessive fertilizer application (FAO, 1999). For example, Augstburger (1989) reported that manure applications should be performed once in a cultivation cycle of three-four years, due to its residual effect. Nowadays, farmers report annual fertilizer applications, and additional fertilization events may also take place upon plant emergence or flowering. Chicken manure management by local farmers is highly variable: from direct use upon delivery to roadside storage in piles during several months, with and without turning of the piles. These practices result in fertilization events with manure at different stages of decomposition, and most likely of different compost quality. As farmers observe variable potato yields, root damage and report seed germination problems, we hypothesized that these different field observations might be due to the varying composting procedures used for the chicken manure applied to the fields. In particular, we hypothesized that fresh manure might present phytotoxic effects, impairing seed germination and plant development and that the application of mature compost would lead to better soil quality. The aims of this study were to evaluate: (1) the effect of turning frequency on chicken manure compost maturation; and the effects of chicken manure compost in different maturity stages produced with or without turning on (2) phytotoxicity for plant germination and growth, (3) soil quality.

5.2 Materials and Methods

5.2.1 Chicken manure composting

Chicken manure was collected two weeks after the removal of animals from a single broiler house of a typical chicken farm of "Asociación de Avicultores Cochabamba ADA" (poultry farmers association of Cochabamba). Manure composting was performed in the field at University Mayor de San Simon (Bolivia). Manure was homogenized and used to establish

9 piles (length, width and height=1.5×1×0.7 m, 0.7 m^3 each), randomly assigned to three different turning frequency treatments, representative of local farmers' practices: no turning of the piles (0T), turning every 4 weeks (4W), and turning every 2 weeks (2W). The piles were placed on plastic sheets to avoid leaching and covered with plastic film to avoid desiccation. Water holding capacity (WHC) was estimated by the Shaw's method (Jenkinson and Powlson, 1976), and manure was adjusted to 60% WHC. The composting process was followed during 150 days. Manure samples were taken every week during the first three months and then every two weeks until the end of the experiment, resulting in a total of sixteen sampling dates.

At each sampling date, the temperature of the piles was recorded and six samples (3 kg each) were collected from each pile (two from the top, two from the middle, and two in the lower part of the pile).). Samples were homogenized and sieved (4 mm mesh), resulting in one composite sample per compost pile. A sub-sample was stored at 4°C and used for physico-chemical and respiration analyses. The remaining sample was air dried in the dark, and stored for the seed germination and plant growth experiments.

Water content was determined by weight difference after drying the samples at 105°C for 48 h. pH was determined in a manure-water solution (1:5 w/v) with a pH-meter (Oaklon PC 700). Total organic carbon was determined with the Walkley-Black wet combustion method. Briefly, 6 g manure were mixed with a solution containing 30 ml distilled water, 6 ml sulfuric acid (98%) and 3 ml potassium dichromate, and titrated with ferroin indicator solution (Cáceres, 2012). The total nitrogen content was determined with the Kjeldahl digestion method. Briefly, 0.3 g manure were digested with 6 ml sulfuric acid (98%), distilled with 25 ml sodium hydroxide 10N and NH_3 collected on a boric acid solution (7%). Finally, the ammonia present in the solution was determined through titration with Tashiro methyl red/Methylen blue indicator and sulfuric acid (0.05%) (Cáceres, 2012). The respiration potential was determined as CO_2-C accumulation during 3 h in the headspace of an amber bottle (360 ml) from 5 g fresh manure at 20°C after overnight pre-incubation (Robertson et al., 1999). Gas samples (4 ml) were taken at 0, 120, 150, 180, and 210 min with an airtight syringe (Hamilton Model 1005) and analyzed with an infrared absorption gas analyzer (EGM-4 PPsystem, UK). The respiration potential was estimated by linear regression of CO_2-C against time. The metabolic quotient (qCO_2, an indicator of the quantity of respired CO_2-C per unit of soil microbial biomass (see below), reflecting maintenance energy requirement) was calculated by dividing the respiration potential by MBC (Anderson, 2003; Anderson & Domsch, 1989, 1990).

5.2.2 Seed germination and plant growth experiments

Manure collected from the nine piles was used for seed germination and plant growth experiments. Six of the sixteen sampling dates were selected to cover the whole duration of the composting process and the three turning frequencies 0T, 4W, and 2W. Selected sampling dates corresponded to 2 (mesophilic phase), 23, 45 (thermophilic phase), 46, 107, and 150 (cooling down phase) days of composting. The effect of chicken manure at different stages of decomposition and with varying turning frequencies on seed germination, plant

growth and soil quality indicators was thus evaluated through a factorial design with the factors compost age (6 levels: 2, 23, 45, 65, 107 and 150 days of decomposition) and turning frequency (3 levels: 0T, 4W, and 2W), their combination resulting in a total of 18 treatments (hereafter referred as "manure treatments").

5.2.2.1 Effects of chicken manure on seed germination

The effects of manure extracts on seed germination were assessed using certified *Avena sativa* L. seeds as plant species indicator, obtained from SEFO-SAM (Semillas Forrajeras Sociedad Anónima Mixta). Seeds were subjected to a pre-test for germination in 10 Petri dishes, containing each 10 seeds, under water saturated atmosphere at 23°C (ISO11269-2:2012). The germination percentage was assessed after 6 days, resulting in 94% germination rate.

Manure extracts were prepared using 5 g dry manure mixed with 50 ml distilled water (1:10 w/v, dry weight basis) and agitated for 1 h at 200 rpm (Gao et al., 2010). Extracts were centrifuged (12000 rpm) and 3 ml were poured in sterile plastic Petri dishes (in triplicate) containing one layer of Whatman #2 filter paper. Ten seeds were placed into each Petri dish and covered with a layer of thin sterile Whatman #2 filter paper to ensure that the whole seed surface was in contact with the solution. For each of the 18 manure treatments (3 turning treatments, 6 sampling dates), three replicate Petri dishes were established, resulting in a total of 54 Petri dishes that were incubated at 23°C for three days. Three control Petri dishes containing seeds and distilled water were included. The number of germinated seeds, and the number of germinated seeds showing apparent root damage in the form of tissue oranging (Supplementary material S1) were recorded.

5.2.2.2 Effect of chicken manure on plant growth

We tested the effect of soil fertilization with manure at different stages of decomposition and with varying turning frequencies on germination and growth of *A. sativa* as plant species indicator, and on soil quality indicators following ISO 11269-2 (2012).

Soil was collected from a 4-year fallow field located in Chullchunqani community (Pocona municipality, Cochabamba) in the central Andean region of Bolivia. Fallow soil was chosen for the experiment, as the use of soil from crop fields may lead to potential residual effect of the last fertilization event, which can last to lasts up to two years (Condori et al., 1997). The 0.4 ha field was divided into 10 m^2 quadrants and ten quadrants were randomly selected. One soil sample was collected (20 cm depth) in the middle of each quadrant and all samples were bulked, homogenized, air dried, and sieved (4 mm).

For each of the 18 manure treatments (3 turning treatments, 6 sampling dates), 2.4 g dry manure of each pile was mixed with 450 g dry soil (0.6%) in triplicate (54 samples in total). The manure:soil ratio was determined through a poll conducted with farmers of the Pocona municipality (n=21) to determine the average quantity of manure applied per hectare. Soil-manure mixtures were adjusted to 80% water holding capacity and placed into plastic pots. Three control pots containing soil without manure, and three technical controls containing

ultra-pure sand (Roth) were also included in the experiment. In each pot, ten *A. sativa* seeds were planted (10-15 mm depth) spaced uniformly.

Pots were randomly placed in a greenhouse and rearranged twice a week during the experiment. Soil humidity was maintained daily by recording pots' weight and adding the required amount of deionized water. The pots containing sand were watered with a nutrient solution (S2 of Supplementary material) to avoid plant nutrient deficiencies. The start of the experiment was determined by the emergence of 50% of seedlings in the control pots (containing not fertilized soil). At this moment, the number of emerged seedlings were recorded, and five seedlings were kept in each pot to reduce potential competition between plants. Seedlings were allowed to grow for 15 days and were then carefully removed from the pots. Root and shoot length of each plant were recorded, all plants were dried (70°C for 48 h) and weighted.

5.2.2.3 Effects of chicken manure on soil characteristics

The remaining soil from the plant growth experiment was stored at 4 °C and analyzed for soil physico-chemical characteristics. Soil pH was determined with a pH-meter meter (HI2550 HANNA instruments) in 15 g fresh soil in a KCl 1M solution (1:2 w/v; Allen, 1989). Water soluble and hot water extractable carbon and nitrogen were determined using a sequential extraction. Water soluble carbon and nitrogen were extracted from 7.5 g fresh soil with 45 ml distilled water at room temperature (Ghani et al., 2003). The soil solutions were agitated (120 rpm, 30 min) centrifuged (4000 rpm, 10 min), and the supernatants were filtered and stored for chemical analyses. The remaining soil was re-suspended in 45 ml distilled water, and placed in an oven at 80 °C for 16 hours to determine the hot water extractable carbon and nitrogen (Ghani et al., 2003). Solutions were agitated and centrifuged as described above. Water soluble carbon (WSC) and nitrogen (WSN), and hot water extractable carbon (HWC) and nitrogen (HWN) of the solutions were determined with a total organic carbon analyzer (Analytik Jena Multi N/C 3100 TOC/TN Analyzer).

Soil microbial biomass carbon (MBC) and nitrogen (MBN) were determined through fumigation-extraction method (Anderson and Domsch, 1978). Fumigation of soil subsamples was carried out for 3 days in a vacuum desiccator with alcohol-free chloroform. Ten grams soil of fumigated and non-fumigated samples were extracted with 50 ml 0.5 M K_2SO_4 (1 h shaking at 180 rpm and filtration through Whatman filter #42). Organic carbon and total nitrogen in the extracts were measured with a total organic carbon analyzer (Analytik Jena Multi N/C 3100 TOC/TN Analyzer). The respiration potential was determined as CO_2-C accumulation during 3h in the headspace of an amber bottle (125 ml) from 20 g fresh soil, at 20 °C in the dark after an overnight pre-incubation (Robertson et al., 1999), as described above.

5.2.3 Statistical analyses

We evaluated initial manure characteristics (2 days of decomposition) of the three turning frequencies 0T, 4W and 2W with one-way ANOVA and Tukey tests on the variables

measured. The effect of turning frequency 0T, 4W and 2W on manure composting were evaluated by fitting linear and non-linear models to the variables measured for each manure pile during the composting period. Linear models were used to fit pH, TOC, TN, and C:N. Negative asymptotic function was used for the respiration potential. General additive models were used for temperature with smoothing function and 5 knots for the explanatory variable days (Zuur et al., 2009). One-way ANOVA and Tukey post-hoc tests were conducted on the slopes of linear models, on the minimum attainable value of respiration potential, and on the maximum average value of temperature.

Changes in seed germination, plant growth and soil characteristics compared to control (not fertilized) soil were assessed through Dunnett's test. Two-way ANOVAs were used to assess the effect of compost age, turning frequency and their interaction. Tukey post-hoc tests were used for assessing the differences between manure treatments.

All analyses were conducted in the R software (R Core Team, 2018), using the packages "car" (Fox et al., 2018), "dplyr" (Wickham et al., 2019), "lsmeans" (Lenth, 2016), "mgcv" (Wood, 2017), "multcomp" (Hothorn et al., 2017), "propagate" (Spiess, 2018), "userfriendlyscience" (Peters, 2018).

5.3 Results

5.3.1 Changes of manure characteristics during composting

There were no significant differences in the initial temperature, pH, TOC, TN, C:N ratio and respiration potential between the compost piles assigned to the three turning frequency treatments (Table 5.1). Temperature, TN, C:N ratio and respiration potential changed over the course the composting process (Figure 5.1). The average temperature of the manure piles at the beginning of the experiment was ca. 40°C. Maximum average temperatures were reached after one month of decomposition, ranging between 45.0 and 46.8 °C, followed by a decrease in temperature until the end of the composting process, when average temperatures reached 31.0°C for the three turning frequency treatments. Total nitrogen content in the manure of piles turned every 4 and 2 weeks decreased at constant rates of -0.07 and -0.08 g kg^{-1} day^{-1}, respectively, while a significantly higher rate was found for 0T treatment (-0.02 g kg^{-1} day^{-1}, slope not significantly different from 0, *p*-value >0.05). The C:N ratio of the manure piles of the 4W and 2W treatments increased linearly at a rate of 0.04 and 0.06 units day^{-1}, respectively, while 0T had significantly lower rate change of 0.01 units day^{-1} (slope not significantly different from 0, *p*-value >0.05). Changes in the respiration potential of the manure piles were assessed by fitting convex asymptotic curves. The initial respiration potentials of 0T, 4W and 2W were 216.9±38.8, 223.5±49.2 and 227.9±43.6 µg $C\text{-}CO_2$ h^{-1} g^{-1}, respectively, and decreased during the first fifty days of decomposition until they reached the minimum attainable respiration potentials according the fitted models of 30.4±3.7, 21.3±2.7 and 14.3±2.2 µg $C\text{-}CO_2$ h^{-1} g^{-1}, with significantly higher values for 0T compared to 2W and 4W respectively.

There were no significant changes in pH and TOC of the manure piles during the composting period (slopes of the three treatments not significantly differ from 0, *p*-value >0.05, Figure 5.1).

Table 5.1 Manure characteristics at the beginning of the composting period (2 days of composting) for the three turning frequencies: 0T= no turning of the piles, 4W= turning every 4 weeks, 2W= turning every 2 weeks. Mean and standard deviation are presented (n=3). Different letters indicate significant differences between treatments (ANOVA and Tukey tests, p-value <0.05).

	0T	4W	2W
Temperature (°C)	40.0±1.0 a	39.9±1.9 a	39.2±0.76 a
Water content (%)	61.6±8.6 a	64.2±1.7 a	65.8±3.0 a
pH_{H2O}	8.8±0.1 a	8.0±0.6 a	8.3±0.9 a
TOC (g kg^{-1})	460.2±9.5 a	419.9±20.5 a	418.3±22.5 a
TN (g kg^{-1})	40.7±4.6 a	37.5±2.6 a	39.1±2.2 a
C:N ratio	11.4±1.5 a	11.2±1.0 a	10.7±0.3 a
Respiration potential (µg C-CO^2 h^{-1} g^{-1})	216.9±38.9 a	223.5±49.2 a	228.0±43.6 a

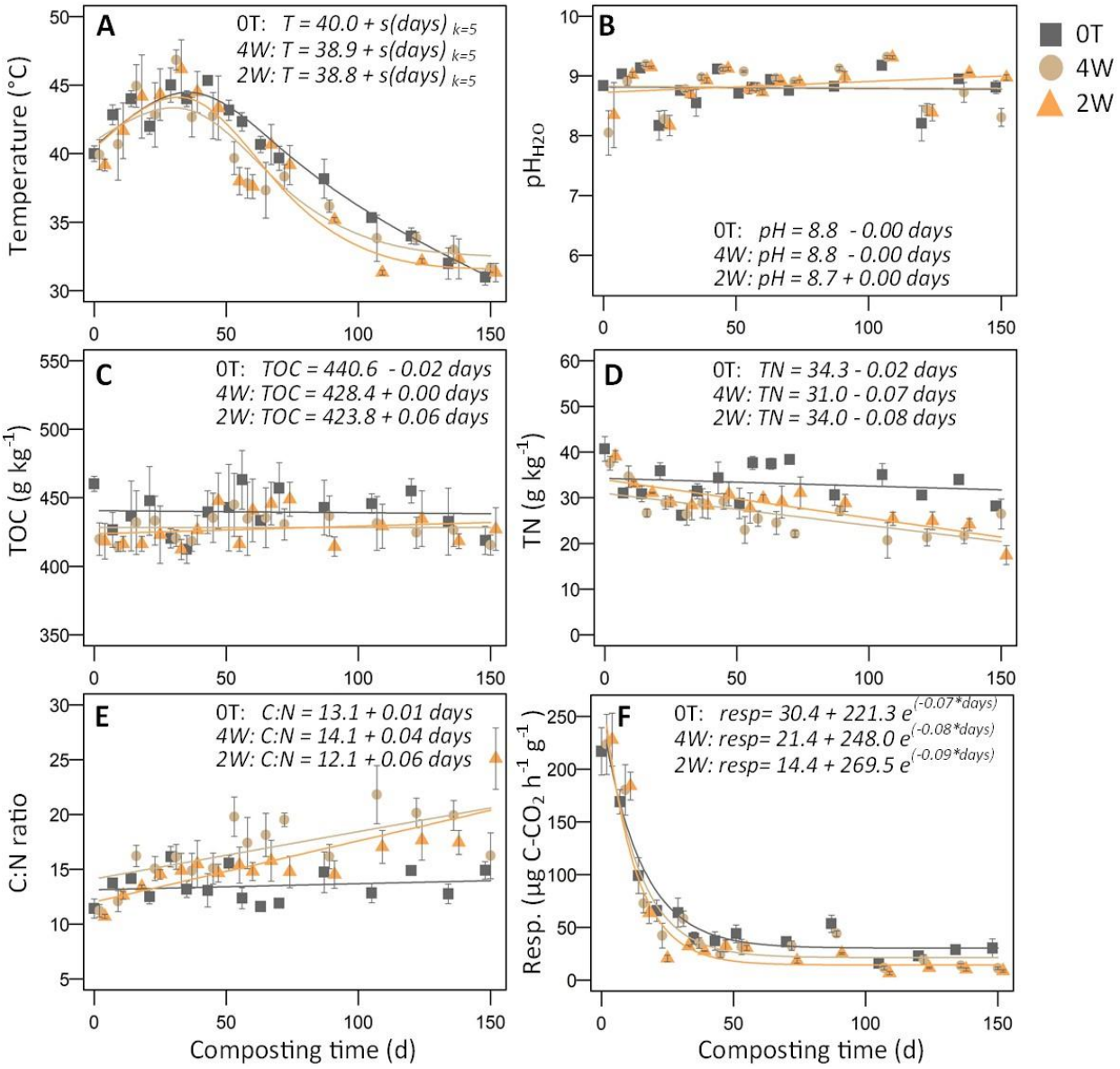

Figure 5.1 Evolution of the manure characteristics (temperature, pH, TOC: total organic carbon, TN: total nitrogen, C:N ratio, and Respiration: respiration potential) over 150 days of composting. For each sampling time we present the mean and standard deviation of the turning frequency treatments: no turning of the piles (0T), turning every 4 weeks (4W), and turning every 2 weeks (2W). General additive models were used for temperature with smoothing function and 4 knots for the explanatory variable days (Zuur et al., 2009). Linear models were used to fit pH, TOC, TN, and C:N. Negative asymptotic function was used for the respiration potential. Each fitted line represents the averaged model (n=3) for each of the turning frequency treatments. General additive models were fit for the evolution of temperature where a smoothing function with 5 knots was applied for "days". Linear models were fit for pH, TOC, TN and C:N ratio, and negative asymptotic model were fit for respiration potential

5.3.2 Effects of chicken manure on seed germination, root damage and plant growth

Water extracts of chicken manure sampled at different composting stages and prepared with different turning frequencies did not have any significant phytotoxic effect on seed germination (Figure 5.2, S3 of supplementary material). Compost age had a significant effect on the percentage of germinated seeds showing apparent root damage (Figure 5.2, S3 of supplementary material). Seeds germinated with extracts of manure (sampled at 2 and 23 days of composting) had the highest percentage of root damage, ranging between 47 and 53%. Seeds germinated on extracts of manure sampled at 150 days of composting had the lowest percentage of root damage, ranging from 9 to 24%. Although the two-way ANOVA test indicated a significant effect of turning frequency on apparent root damage, Tukey test did not detect significant differences between turning frequency treatments.

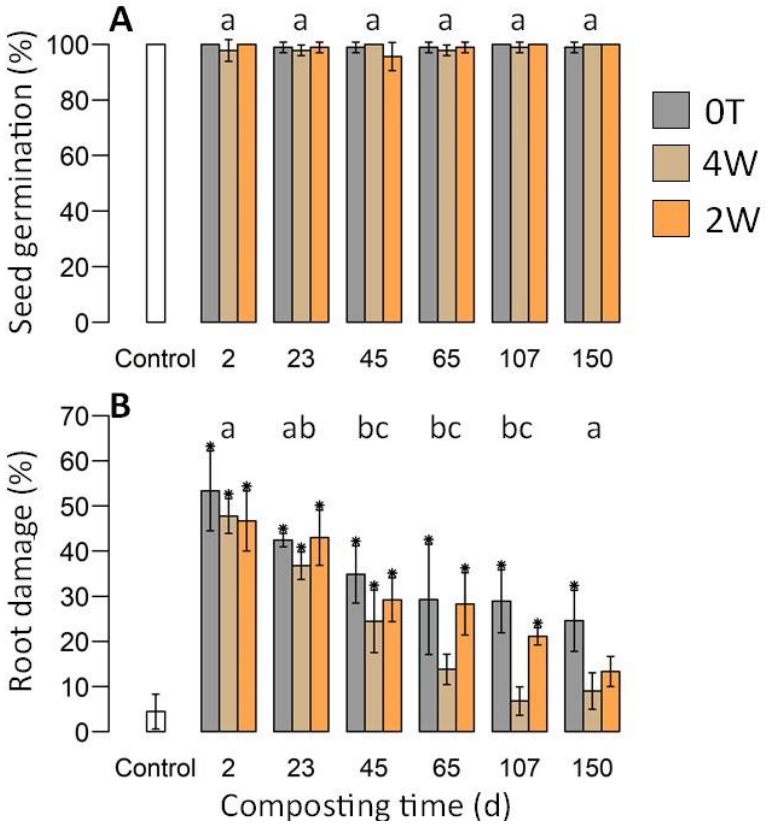

Figure 5.2 Effects of chicken manure extracts at different stages of decomposition (2, 23, 45, 65, 107 and 150 days of composting) and with different turning frequencies (0T: no turning of manure piles, 4W: turning every 4 weeds, 2W: turning every 2 weeks) on seed germination and apparent root damage. For each treatment the mean and standard deviation are presented (n=3). Asterisks indicate significant difference in comparison to control seeds germinated in water (Dunnett's test, p-value <0.05). Different letters indicate a significant differences between compost age (two-way ANOVA and Tukey, p-value <0.05).

Percentage of seeds germinated in manure extracts did not differ from the percentage of seeds germinated in water. Seeds germinated in manure extracts had significantly higher percentage of apparent root damage in comparison to seeds germinated in water for the

fifteen manure treatments. Only the extracts of manure sampled at 65 and 107 days of composting turned every 4 weeks did not significantly differ from seeds germinated in water, as well as extracts of manure sampled at 150 days of composting turned every 2 and 4 weeks (Figure 5.2).

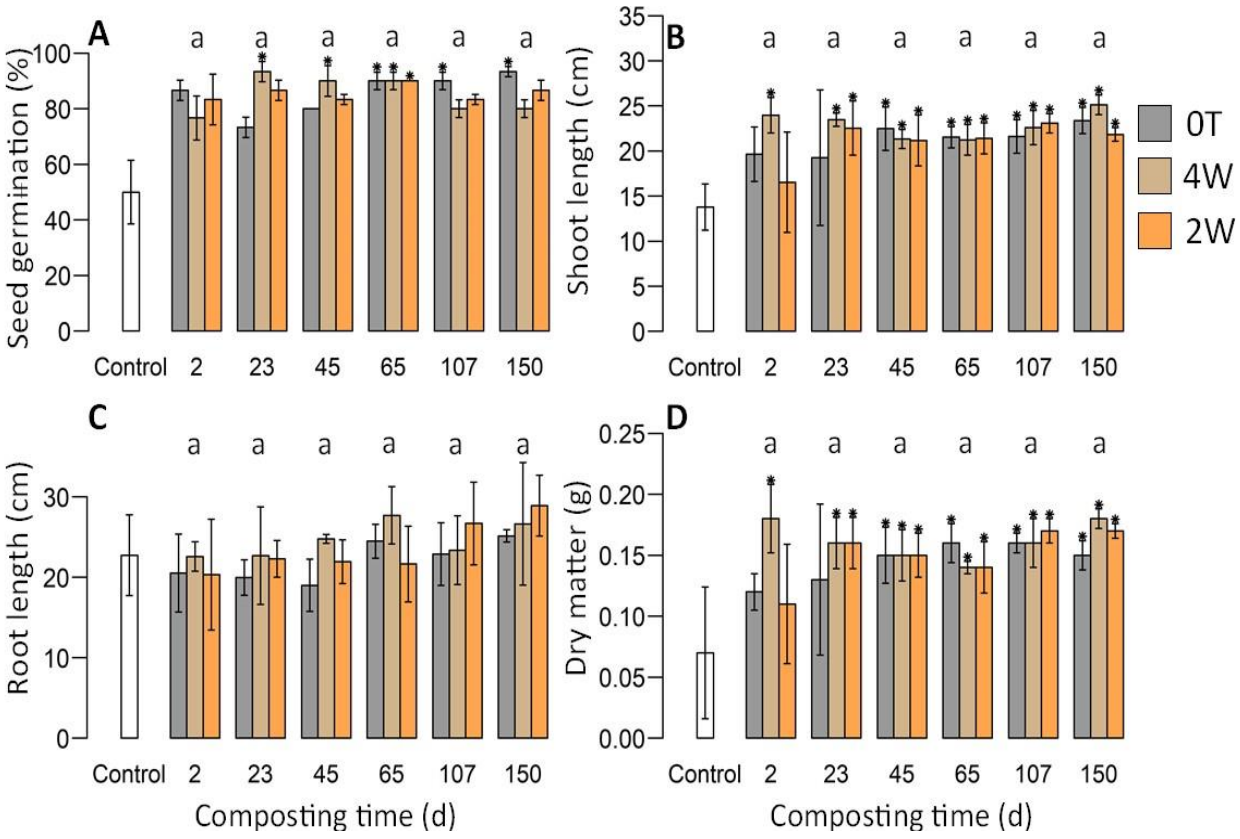

Figure 5.3 Effect of chicken manure fertilization of soils on seed germination, shoot and root lengths and dry matter of A. sativa root and shoots. Manures were at different stages of decomposition (2, 23, 45, 65, 107 and 150 days of composting) and prepared with different turning frequencies (0T: no turning of manure piles, 4W: turning every 4 weeds, 2W: turning every 2 weeks). For each treatment the mean and standard deviation are presented (n=3). Asterisks indicate significant difference in comparison to not fertilized control soil (Dunnett's test, p-value <0.05). Different letters indicate a significant differences between compost age (two-way ANOVA and Tukey tests, p-value <0.05).

Fertilization of soil with chicken manure of different compost age and prepared with different turning frequencies did not have any significant effect on seed germination, shoot length, and biomass production of *A. sativa* (two-way ANOVA, *p*-value >0.05, Figure 5.3). Though two-way ANOVA indicated that compost age had a significant effect on root length (*p*-value =0.04), Tukey post hoc comparison did not detect differences between manures sampled at different stages of composting (Figure 5.3). In comparison to the control, non-fertilized soil, the addition of manure to the soil had a significant effect on seed germination, shoot length and plant biomass ("dry matter"). Seven treatments that received manure showed higher number of germinated seeds in comparison to control soils. *A. sativa* plants grown in soils that received manure sampled along the composting process (from 2 to 150

days of composting) had longer shoots and produced significantly more biomass in comparison to plants grown in non-fertilized soils. The addition of manure did not affect the root length in relation to plants grown in control soil.

5.3.3 Effects of chicken manure on water soluble C and N and respiration potential

The addition of chicken manure of different compost age and prepared with different turning frequencies showed a significant interaction effect on WSC content and soil pH_{KCl} (two-way ANOVA, *p*-value <0.05, S3 of supplementary material and Figure 5.4). Comparison between soil treatments fertilized with manure of different composting time indicates that the lowest WSC values were recorded at 2 days of composing and the highest values were recorded after 65 days of composting, whereas no differences were observed for soil pH_{H2O}. Compost age had a significant effect on MBC and MBN (two-way ANOVA, *p*-value <0.05), however Tukey tests did not detect differences between soils fertilized with manure of different compost age (Figure 5.4). Turning frequency was significant for MBC and MBN, with the manure piles turned every 2 weeks showing the highest values MBC and MBN values (S4 of supplementary material). For respiration potential, turning frequency was also significant but Tukey test did not detect differences between turning frequency treatments (S4 of supplementary material). Compost age did not had a significant effect on respiration potential. Fertilization of soil with chicken manure of different compost age and prepared with different turning frequencies did not have an effect on WSN, HWC and HWN (two-way ANOVA, *p*-value >0.05, Figure 5.4).

In comparison to control soil, the addition of chicken manure lead to a significant increase in HWC of eight treatments (combinations of turning frequency and compost age; Figure 5.4). HWN also increased in comparison to control soil, except for the soils receiving of treatment 0T at two and 45 days of decomposition, and the soil receiving manure of treatment 2W at 150 days of decomposition. The addition of manure sampled at 2 and 23 days of composting resulted in significantly higher MBC and MBN in comparison to control soils. Similarly, the addition of chicken manure had a significant effect on respiration potential. For the majority of the incubation treatments respiration potential increased in comparison to control non fertilized soils, except for 0T and 2W treatments at 45 days of decomposition, and the 2W at 150 days of decomposition.

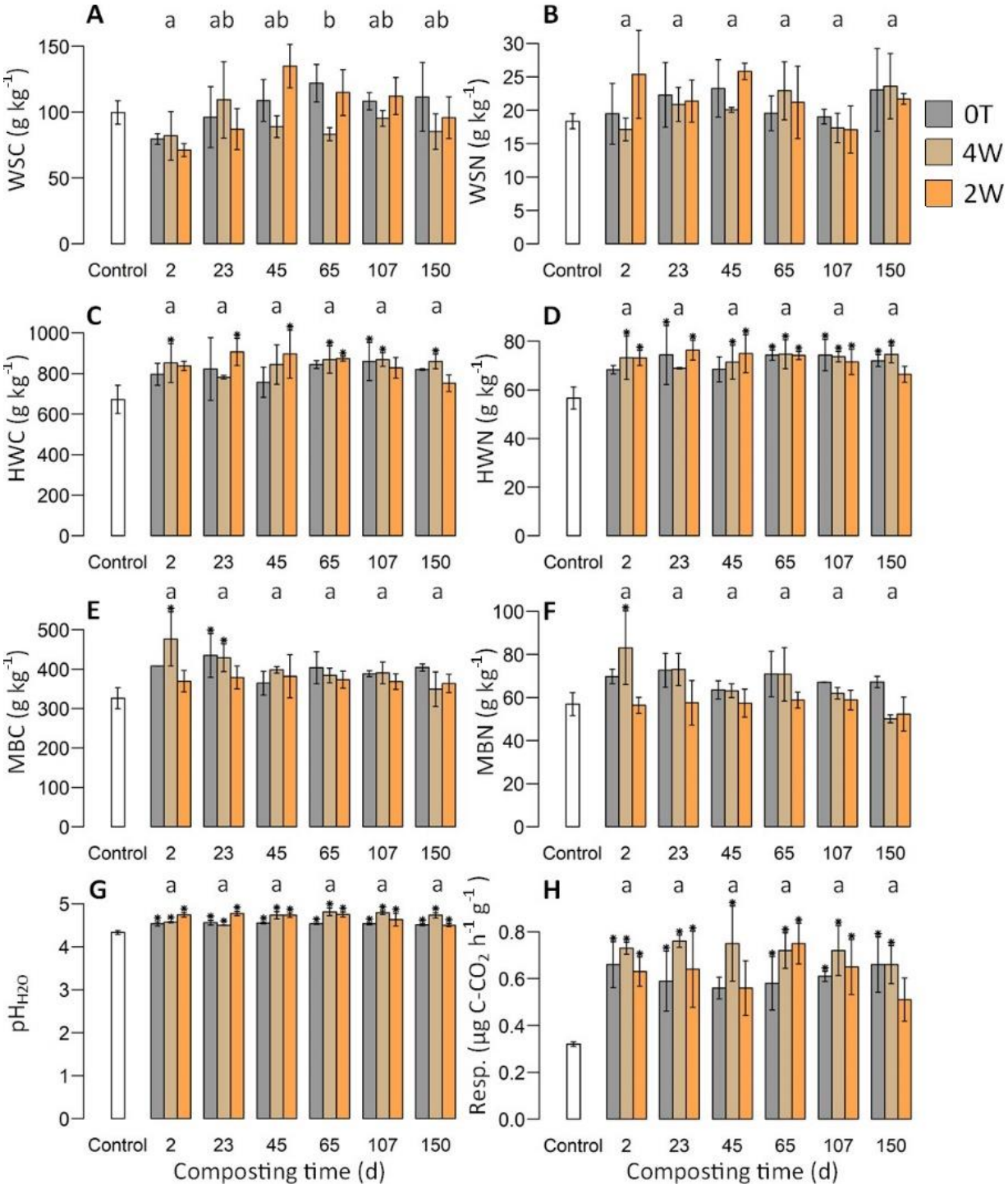

Figure 5.4 Effect of fertilization with chicken manure on water soluble carbon (WSC), water soluble N (WSN), hot water extractable C (HWC), hot water extractable N (HWN), microbial biomass C (MBN), microbial biomass N (MBN), pH and respiration potential (Resp.). Manures were at different stages of decomposition (2, 23, 45, 65, 107 and 150 days of composting) and prepared with different turning frequencies (0T: no turning of manure piles, 4W: turning every 4 weeds, 2W: turning every 2 weeks). For each treatment the mean and standard deviation are presented (n=3). Asterisks indicate significant difference in comparison to not fertilized soil (Dunnett's test, p-value <0.05). Different letters indicate a significant differences between compost age (two-way ANOVA and Tukey tests, p-value <0.05)

5.4 Discussion

5.4.1 Effects of turning frequency on compost stability

We observed the four phases of composting based on changes in compost temperature: mesophilic, thermophilic, cooling down, and maturation phase (Fogarty and Tuovinen, 1991). A temperature between 50 and 55 °C is required for elimination of pathogens, however, in our study the highest average temperatures ranged between 45 and 47 °C, and may not be high enough for proper manure sanitation. Nevertheless, improper manure sanitation may not lead to potato diseases because the soil-borne pathogens reported in this agricultural region (nematodes *Nacobbus aberrans*, and *Globodera* spp.; Franco et al., 1999) have not been linked to chicken manure fertilization. On the contrary, chicken manure applications may restrict their development and reduce their abundance in soils (Cristobal-Alejo et al., 2006; Rodríguez-Kábana, 1986). After 150 days of composting, turned and non-turned compost piles did not show any difference in the final temperature. Temperature stabilization after 150 days under all treatments indicated that the end of the bio-oxidative decomposition had been reached, and that the compost was stable (Iglesias Jiménez and Perez Garcia, 1989). However, the temperature decrease of piles turned every 4 or 2 weeks was more rapid than without turning, and stabilized already after 100 days of composting, indicating faster manure stabilization. This is in accordance with previous studies showing that turning of compost piles accelerates decomposition by improving aeration, and consequently microbial activity (Khalil and Hassouna, 2013).

Despite the fact that temperature is a practical measurement for following the composting process, it lacks precision for assessing manure stabilization due to variability in heat loss (Willson and Dalmat, 1986). In contrast, microbial respiration provides a direct indication of the quantity of easily degradable organic compounds in the manure, and high respiration rates are associated with the lack of compost stability (Gao et al., 2010; Willson and Dalmat, 1986). In our study, the respiration potential of the manure piles decreased rapidly during the first month of decomposition, indicating the degradation of most labile organic matter. Manure piles then reached the minimum attainable respiration potential, which suggests manure stabilization (Gao et al., 2010). The lower minimum attainable respiration potential rates reached in the turned piles (Figure 5.1F) indicated that turning events led to a more efficient composting process. Mechanical disruption of the piles during turning events likely increased accessibility of labile carbon to microorganisms and promoted aerobic conditions that favored microbial activity and organic matter decomposition. Similar results have been previously reported, and compost aeration was suggested to play a central role in manure stabilization and reduction of compost phytotoxic effects (Carballo et al., 2009; Nada, 2015). Concomitantly, we observed a significant reduction of root damage of seeds germinated in turned piles compared to non-turned piles (see below). The practice of turning of chicken manure piles should be promoted to reduce the risk of phytotoxic effects on crops or alternatively delay the use of manure that has not been turned.

We observed a decrease in total nitrogen concentrations of turned compost, without a change in total carbon concentrations, during the 150 days of composting, leading to an increase of the C:N ratio. Commonly the C:N ratio of composted material tends to decrease over time (Khalil and Hassouna, 2013), but we observed the opposite trend in the turned piles, which could be due to increased evaporation of NH_3 in this alkaline environment (de Guardia et al., 2008). Estimates indicate that up to 20% of the total of N loss from chicken manure is released to the environment in the form of ammonia (Dróżdż et al., 2020). Also, intense microbial activity could have led to anoxic microsites, favoring gaseous N losses through denitrification (Hao and Chang, 2001).

5.4.2 Effect of chicken manure on seed germination and plant growth

Effects of chicken manure were tested with compost sampled during the mesophilic, thermophilic, cooling down, and maturation phases. The application of chicken manure at different stages of composting to soils, at a rate typically applied by the local farmers, did not lead to differences in seed germination, root and shoot length. Extracts of chicken manure at different stages of composting also did not show any effect on seed germination, but revealed a decreasing trend in seedling root damage (oranging) with increasing compost age. Tissue oranging is generally attributed to cation-anion imbalances in the plant, to rhizosphere acidification, and it has been widely reported in plants exposed to high ammonium/ammonia concentrations (above 0.1 to 0.5 mmol of NH_4^+ per L^{-1}; Britto and Kronzucker, 2002; Westwood and Foy, 1999). Root damage decreased with compost age, indicating compost maturation, likely due to the decrease of NH_4^+/NH_3^- or to the degradation of potential phytotoxic compounds, such as organic acids and phenols (Riffaldi et al., 1986). Moreover, turning of the piles reduced apparent root damage to similar levels than those recorded in control, non-fertilized, soils after 64 days of composting, indicating faster compost maturation associated to this practice. These results are in accordance with previous studies indicating faster decrease of phytotoxicity for aerated composts (Carballo et al., 2009; Riffaldi et al., 1986). High amount of salts in manure extracts may also contribute to the observed effects on roots, associated to osmotic and ionic stress caused by high concentrations of Na^+, Mg^{2+}, Cl^-, and SO_4^{2-} (Baligar et al., 1998; Li-Xian et al., 2007). Although salinity of manure has been reported to affect seed germination (Delgado et al., 2010), we did not observe effects on percentage of germinated seeds.

We did not observe a decrease in root length or plant biomass when the compost at different stages of maturation was applied to the soils. This could be due to a dilution effect upon soil fertilization, to the adsorption of phytotoxic compounds to soil organic matter or to their degradation by soil microorganisms (Xiao et al., 2020). In the field, however, manure applications are not homogeneous, and furrow fertilization (chicken manure applied in direct contact with potatoes at sowing time) is a common practice that may lead to locally high application rates. Furthermore, farmers report that additional fertilization events may take place, leading to excessive fertilization (FAO, 1999). The farmers' rationale is that increased manure application would lead to higher potato yields, however, higher manure additions are not coupled with increased crop yield (Hoover et al., 2019), and could increase the risk

of over-fertilization instead. The risk of phytotoxic effects may thus increase and lead to the root burning effect reported by farmers. Furrow fertilization should be avoided to reduce the risk of root damage in crops. Soil nutrient testing to asses optimal fertilization regimes based on plant needs is required to reduce the risk of negative effects associated with over-fertilization, such as NH_4^+toxicity and contamination of groundwater with NO_3^- and trace elements (Dróżdż et al., 2020), and to reduce production costs.

Despite the phytotoxic effect caused by manure extracts, we observed a positive effect of soil fertilization with chicken manure on plant development (seed germination, shoot length, biomass production). Improved plant development in fertilized soils indicated that manure addition contributed to soil quality, likely by increasing organic matter, micronutrient contents, reducing soil acidity, and improving water availability to plants (Mann et al., 2002; Mondo et al., 2012).

5.4.3 Effect of chicken manure on soil C and N fractions and respiration potential

Fertilization of soil with chicken manure increased the amounts of hot water extractable carbon and nitrogen. These fractions are considered to provide readily available nutrients for plants and microorganisms (Chodak et al., 2003; Curtin et al., 2021), and they likely contributed to the higher microbial activity observed. These results are in accordance with previous studies indicating that fertilization increases labile carbon fractions (Darby et al., 2006). Due to the close link between labile pools and soil fertility, increases in the labile hot water extractable C and N are indicative of increased available nutrients to plants (Anderson and Domsch, 1980; Ghani et al., 2003), and therefore improved soil quality for cultivation. Moreover, soils with high level of labile organic matter have complex soil microbial communities that could suppress the activity of soil-borne pathogens and promote plant growth (Aryantha et al., 2000).

Microbial biomass increased in soils fertilized with manure at mesophilic and thermophilic phases of decomposition (2 and 23 days of composting), which is likely explained by the labile organic compounds present in not stabilized manure that promoted microbial growth. Surprisingly, the use of manure at mesophilic and thermophilic composting stages did not lead to higher soil respiration potential rates. This could be due to changes in the energy maintenance demands of soil microorganisms and C allocation in structural compounds rather than respiration, however, we did not observe differences in the quantity of respired CO_2-C per unit of microbial biomass (an indicator of energy maintenance demands; Anderson and Domsch, 1989, data not shown). As the decomposition process advances, labile organic compounds present in chicken manure are degraded by microorganisms, and the effect on microbial biomass decreases. On the other hand, respiration potential increased in response to manure fertilization independent of compost age, suggesting that this measurement is more sensitive to C additions than microbial biomass. Also the short-term response of soil microorganisms to labile organic inputs (Poll et al., 2007) could have influenced the detection of differences between treatments. It is thus possible that the microbial biomass could have increased after fertilization and then gradually decreased during the plant growth experiment. Additional factors may also have contributed to the

observed response of soil microorganisms to manure additions, such as the microbial community composition or ecological interactions with plants and nematodes (Faissal et al., 2017; Liang et al., 2020; Poll et al., 2007).

The decrease in soil acidity could be due to the high pH of the manure added to the soil, but previous studies also indicate that manure applications increase soil organic matter, which acts as a pH buffer and increases nutrient contents (Mann et al., 2002; Mondo et al., 2012; Soremi et al., 2017). Less acidic conditions may favor plant growth, particularly potato production, since drastic potato yield reductions have been reported pH_{H2O} values below 4.6 (Lierop et al., 1982). Altogether these results suggest that different management practices of manure composting process (turning events and compost age) did not lead to significant differences in labile C and N fractions, microbial activity and soil pH when used for fertilization. However, the use of not stabilized manure should be avoided, as immature manure increases the risk of phytotoxic effects on plants.

5.5 Conclusions

Chicken manure piles subjected to turning events showed an acceleration of the decomposition process, more efficient stabilization and faster maturation. Chicken manure fertilization had a positive effect on soil quality and plant development. Fertilization increased hot water extractable C and N, respiration potential, and soil pH compared to non-fertilized soil, but the use of manure at different stages of decomposition did not lead to significant differences on soil properties. Concomitantly, seed germination, shoot length, and biomass of *A. sativa* increased compared to non-fertilized soils. Nevertheless, we observed phytotoxic effects of extracts from immature manure on *A. sativa* roots, which significantly decreased in chicken manure piles subjected to turning events. A minimum of 100 days of decomposition and turning manure piles every four weeks is thus suggested as composting practice to ensure proper manure stabilization, maturation. Application of manure at earlier stages of decomposition should be avoided to reduce the risk of phytotoxic effects caused by immature manure.

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Author contributions

Alejandro Coca-Salazar and Monique Carnol designed the study and wrote the manuscript. Data collection, material preparation, and data analyses were performed by Alejandro Coca-Salazar. Both authors commented, read and approved the final manuscript.

Chapter 6

General discussion and conclusions

Major drivers of land-use changes in the world are agricultural intensification and increased food demand. Due to the central role of soils in ecosystems, modifications of their physico-chemical or biological characteristics caused by land-use changes and intensification may have cascading effects on soil functioning and the provision ecosystem services. Soil quality evaluations are tools to determine responses of soils to changes and to guide sustainable management practices (Karlen et al., 2003). Assessment of soil quality, however, should go beyond the measurement of a set of properties and processes, and must include their interactions and responses to changing conditions (Bünemann et al., 2018). Information provided by the links between indicators contributes to understanding the multi-directional interactions between the physical, chemical and biological soil components, essential for guiding management practices. We investigated the combined response of soil physico-chemical properties, microbial processes and their interactions as indicators of soil quality of fields cultivated with potato, short fallow fields (2-6 y) and eucalyptus plantations. This integrative approach contributed to a better understanding of the responses of soils to land-use changes, which is essential for guiding sustainable management practices.

Despite the fact that soil quality has been widely studied in Europe, Asia and North America, to our best knowledge soil quality evaluations of agricultural ecosystems have not been conducted in the Andean region. Moreover, soil research in Bolivia traditionally focused on soil fertility assessments (Cáceres, 2012; Ministerio de Medio Ambiente y Agua, 2014; Orsag et al., 2011; Pacasa-Quisbert, 2017; Silicuana et al., 2018). This study thus presents the first information of soil quality of the main land uses of potato-based agricultural ecosystems in the Central Andes and Bolivia.

6.1 Soil quality of agricultural fields: the effects of short fallow periods and fertilization

Fallowing soils over long periods (>10 y) was considered of ecological and economical importance for agricultural production in the Central Andes, as farmers historically depended on this practice for agricultural production (Duchicela et al., 2013; Pestalozzi, 2000; Sivila and Angulo, 2006; Sivila and Hervé, 1994). Improvement of soil chemical, biological and structural quality are generally linked to an increase in nutrient contents, microbial abundance and activity, aggregate stability and abundance of bigger aggregates. We did not observe such changes as a result of fallowing soils, suggesting that the current practice of short fallow periods (<6 y) is insufficient to restore soil quality for cultivation.

The decrease of water soluble N, extractable P, and N transformations as a result of fallowing was likely due to the absence of fertilization, suggesting rapid reduction in N availability to microorganism and plants. The success of long fallow periods to restore fertility and increase potato yields has been explained by the natural plant succession and the establishment of diverse plant communities in fallow fields (Gomez-Montano et al., 2013; Pestalozzi, 2000; Sivila and Angulo, 2006; Sivila and Hervé, 1994). In fact, the idea that a vegetation cover improves soils over the years has been largely described in the ecological literature (FAO, 2006; Oosting, 1942). In Andean ecosystems, the presence of shrubs such as *Parasthephia* spp., *Baccharis* spp. in fields with more than 10 years in fallow contributes to soil organic matter accumulation, increases the abundance of nitrogen-fixing bacteria (*Bradyrhizobium*)

and soil fungi, and reduces the risk of soil erosion (Gomez-Montano et al., 2013). In contrast, in fields under short fallow periods, shrubs are rare and small (Gomez-Montano et al., 2013), and may not significantly contribute to organic matter accumulation, to the establishment of diverse active microbial communities, or to soil erosion control (Jacobsen, 2011). Given that six years is the maximum length of fallow in intensified agricultural communities, plant species that require longer time for seed production may be eventually eliminated. Species with faster life cycles could thus dominate the plant communities, increasing the risk of weed infestation of cultivated fields as previously reported in intensified agricultural areas (FAO, 2006; Petit et al., 2011). The absence of non-crop resources, such as the above mentioned shrubs, represents a loss of secondary outcome from fallow fields under short rotation cycles (Dalle and de Blois, 2006; Vidaurre et al., 2006). Since shrub species have been historically used as firewood, their absence explains the farmers' interest for introducing exotic species (e.g. eucalyptus) as alternative non-crop resources.

We presented evidence that changes in the soil microbial biomass and microbial activity are linked to changes in aggregate size distribution and stability. These results are in accordance with studies suggesting that the formation of organo-mineral complexes and soil organic matter stabilization is driven by microbial activity (Cotrufo et al., 2013; Liang et al., 2017). Consequently, factors affecting the microbial communities would indirectly influence the formation of aggregates. Given that microbial biomass and respiration potential did not change as a result of fallowing soils, the binding agents associated to soil microorganisms would not be affected by this practice. This is coherent with the absence of differences in aggregate size distribution and stability between fields cultivated with potato and fallow fields. Accordingly, Duchicela et al. (2013) reported that at least ten years of fallow are required to detect changes in soil structure. These results suggest that soil structural quality for cultivation was not improved a result of fallowing. If alternative management practices are not applied to maintain soil structural quality, it may decline over the years and negatively impact agricultural production and farmers' incomes.

Authors have warned about the risk of loss of soil fertility and soil erosion associated to agricultural intensification (Godoy, 1984; Zimmerer, 1993a), but managing these problems is not part of the debate among farmers or regional policy makers yet. Moreover, the existing national programs for soil management do not incorporate clear politics for investigating soil responses to management or the implementation of soil conservation strategies at regional or national scales (Murillo and Sahonero, 2003; Viceministerio de Recursos Hídricos y Riego, 2009). So far, some studies have proposed the plantation of native shrubs in fallow soils to improve the fallow fields and reduce the risk of soil erosion (Gomez-Montano et al., 2013; Sims et al., 1999), but these recommendations have not been implemented beyond the agricultural communities where the studies were conducted. Implementation of soil management practices should be integrated in national soil conservation programs that support and monitor their application (Eni, 2012), particularly because farmers might be reluctant to accept conservation technologies and prefer to allocate their resources in activities that ensure immediate economic benefits (Uzeda, 2005).

Currently, fallowing fields for more than 6 years is not a feasible alternative in intensified agricultural areas. Consequently, farmers have incorporated the use of chicken manure as an alternative practice to improve soil fertility. First studies acknowledging the potential of chicken manure were reported in 1980s (Augstburger, 1989, 1983), and today it constitutes the main fertilizer for potato production. We observed that chicken manure fertilization increased the labile C and N fractions of the soils, promoted microbial activity and reduced soil acidity, suggesting a positive effect on soil fertility, as previously reported (Peñaloza et al., 2019). Fertilization also improved seed germination and plant growth, and this effect was independent of chicken manure composting management (frequency of manure turning events and composting time). These results suggest that use of chicken manure for soil fertilization contributed to soil quality for cultivation and might counterbalance the effect of agricultural intensification. Nonetheless, phytotoxic effects (root damage in the form of tissue oranging) were observed for *Avena sativa* seedlings germinated in extracts of immature chicken manure. This effect could be due to cation and anion imbalances in the plant associated with high N contents, but may also be due to the presence of organic acids and phenols. Phytotoxicity decreased after 64 days of composting of manure subjected to turning events, indicating the effectiveness of this practice to accelerate compost maturation. Commonly soils are fertilized once at the beginning of each cultivation cycle, but additional fertilization events may take place according to farmers' judgement, which could lead to over-fertilization, NO_3^- leaching and groundwater contamination (FAO, 1999). Coupled with the common practice of furrow fertilization (manure is applied in direct contact with the potato seed), the risk of manure phytotoxicity increases. In fact, farmers have reported burning of roots and sprouts with consequent reduction of potato yields. A minimum of 100 days of composting and conducting turning events once a month are thus suggested to ensure manure stability and maturity, and reduce potential negative phytotoxic effects on crops. Further field studies are required to determine optimum chicken manure application rates, residual fertilization effects, and the quantity of nutrients lost by lixiviation.

The increased net N mineralization and nitrification rates observed in fields cultivated with potato could be due to the effects of manure (increase of soil N availability and pH). For example, in potato crops, the relative contribution of AOB to total potential nitrification increased up to 36%. This increase indicates favorable conditions for AOB nitrification in fields cultivated with potato, likely as a result of N fertilization. We demonstrated that ammonia-oxidizing archaea (AOA) dominated over bacteria (AOB) in the three land uses, and that changes were correlated to changes in soil acidity acidity and a change in the relative abundance of AOB. Consequently, management practices that increase soil pH (e.g. fertilization with chicken manure) would favor AOB with potentially higher nitrification rates, while soil acidification (e.g. under eucalyptus plantations) would lead to stronger AOA dominance. Moreover, our results indicate *amoA* gene abundance measurements alone are not sufficient for inferring AOB and AOA activity, and their role for nitrification should be determined by separately measuring their nitrification rates.

6.2 The effects of eucalyptus plantations on soil quality

Accumulation of organic matter and increase in soil C have been reported to take place over short time periods after the plantation of eucalyptus (3 years; Bai & Blumfield, 2015). Accordingly, soil organic matter and labile C fractions increased under this tree in our study, and, combined with the absence of disturbance events, an active microbial community was established, which in turn contributed to the formation of bigger and more stable aggregates around fresh plant material and small soil particles. Moreover, aggregate turnover would also slow down compared to agricultural land uses (Kandeler et al., 1999), contributing to C stabilization and sequestration in the smaller aggregates (<53 µm). These results suggest improvement of soil structure, porosity, water and nutrient retention capacity in response to eucalyptus plantation.

Higher organic C content and improved soil structure have been associated with improved of soil quality for cultivation and with favorable physico-chemical conditions for plant growth (Bronick and Lal, 2005). However, other soil changes seem to counterbalance these positive effects under eucalyptus. For example, increases in soil exchangeable Al content and allelopathic effects of the organic compounds produced by this tree may have toxic effects and cause stress to plants and soil microorganisms (Kinraide, 1991). Consequently, net N transformations were strongly affected by the plantation of eucalyptus in our study: net N mineralization and net nitrification decreased, and a strong effect on the potential nitrification on ammonia oxidizing bacteria was observed, drastically reducing their activity. These results suggest that N could be a limiting nutrient for plants and potentially affect agricultural production since farmers report reduced yields in border areas or when potato is cultivated in fields previous planted with eucalyptus. These results are in accordance with reports indicating that nutrient imbalances and allelopathic effects of eucalyptus negatively affect agricultural production (Babu & Kandasamy, 1997; López-poma et al., 2020; Zhang & Fu, 2010). Despite the positive effect of eucalyptus land use on soil organic matter content, C accumulation and soil structure, our results suggest that eucalyptus did not contribute to improvement of overall soil quality for cultivation.

The introduction of eucalyptus plantations to the Andean agricultural regions dates back to the colonial period of the country but its use for extensive afforestation programs started around 25 years ago. Its introduction was considered as a sustainable strategy for economic diversification of communities that historically relied on potato production (Patiño, 2014). Given that eucalyptus contributes to the economic resilience of agricultural communities by providing an alternative economic income, private and governmental programs still subsidize the plantation of this tree (Fonabosque, 2021b; Villalobos, 2020). Moreover, due to its low maintenance costs, high rates of seedlings survival, and the economic potential once trees reach a productive age (approximately 10 years), farmers prefer eucalyptus to alternative native tree species for afforestation (e.g. *Polylepis* spp.). Despite the apparent economic benefits of planting eucalyptus, its use in afforestation programs should be avoided in agricultural regions where potato production is the main economic activity. Careful land-use planning is essential for the establishment of new tree plantations that do

not interfere with cultivation of crops in order to optimize land-use efficiency (Grau and Aide, 2008). Moreover, government policies should be reviewed and if necessary modified so that they encourage sustainable land use rather than practices reducing soil quality of agricultural areas (Eni, 2012).

6.3 Potential indicators of the changes in soil quality

Among the several characteristics that need to be considered when selecting soil quality indicators, a high discriminant power and a relation to a specific soil function or ecosystem service are considered mandatory (Bünemann et al., 2018; Krüger et al., 2017). Our results indicate that the hot water extractable C (HWC) and microbial biomass were sensitive to detect the change from soils cultivated with potatoes to eucalyptus plantations. Moreover, the HWC was highly related to soil respiration potential and net N mineralization, indicating its close link with soil functions related to C and N cycling. Similarly, the microbial biomass was closely related to changes in aggregate stability and size distribution, suggesting a central role of the microbial-derived substances in aggregate formation. Changes of these labile C fractions in response to land use or soil management practices are thus highly informative, and should be considered for soil quality evaluation programs. In fact, they have been successfully included as indicators of changes in soil quality in different long-term soil monitoring programs in agricultural and forested areas of several countries (Bastida et al., 2008; Fauci and Dick, 2015; Merrington, 2006; Rutgers et al., 2009).

Water soluble N, extractable P, and the microbial processes net N mineralization and potential nitrification were sensitive to detect the conversion from potato to fallow land use. Moreover, hot water extractable C and N, as well as respiration potential were also a highly sensitive variables that responded to the addition of chicken manure to soils. In comparison to the chemical properties, the microbial processes (respiration potential, N mineralization and nitrification) provided valuable information about the microbial activity, C and N cycle and nutrients supply to plants. Microbial activity measurements could be used for assessing effects of land-use changes and agricultural practices. Depending on the specific objectives of soil monitoring programs, the inclusion of microbial processes as part of minimum data sets for soil quality evaluations provides valuable information on soil functions related to C and N cycling and organic matter decomposition (Gregorich et al., 1994).

6.4 Future perspectives

Analyses of the results of this thesis opened new questions about potential studies that might contribute to a better understanding of the agricultural ecosystem studied. For example, it is well known that plants exert strong effects on the chemistry of their near soil environment (Cavalcante et al., 2012; Yarzábal et al., 2017), and eucalyptus and potato have been reported to affect the structure of microbial communities beyond their rhizospheres (Cavalcante et al., 2012; Sturz et al., 1998; Zhang & Fu, 2009). Consequently, these plant species could largely determine the composition of the soil microbial community, and their responses to changes. For example, soil processes such as nitrification or organic matter decomposition may be affected not only by environmental factors but also on the microbial

community structure (Gomez-Montano et al., 2013; Zhang et al., 2017). Evaluation of the microbial community composition could thus provide a better insight in how soil microorganisms respond to land-use changes, and how those changes influence soil processes.

Furthermore, allelopathic effects of eucalyptus on soil microorganisms and plants may take place in this study, as indicated by the reduced microbial activity and inability to cultivate potato after eucalyptus reported by farmers. Evaluations of these interactions, however, have not been conducted in the region, and the effects of organic compounds produced by eucalyptus on microorganisms and potato crops remains unknown. Evaluation of the economic impact of eucalyptus on crops, and particularly on potato yields is of particular importance since this exotic tree is present in most of the agricultural communities in the Central Andean region of the country.

In this study, the risk of nutrient losses through leaching and runoff has been suggested to take place in potato crops and fallow soils. Due to the characteristic mountainous topography of the Central Andes, losses of nutrients by leaching could be an important factor determining nutrient balance of agricultural soils. Further, groundwater contamination may result from leaching of fertilizers, due to excessive applications. We underline the need to study nutrient inputs and outputs to contribute to the understanding of mountainous agricultural ecosystems, and to contribute to the management of soil fertility of agricultural soils in the Andes. Consequently, determination of optimal fertilization regimes aiming at efficient use of fertilizers is essential to reduce the risks of over fertilization (nitrate leaching, ground water contamination), as well as to control the production costs.

Improvement of agricultural production in the Andean region also requires the implementation of soil monitoring programs at regional or national scales. It is acknowledged that the study area does not cover the entire geomorphological variability of the Andean region. Consequently, extensive sampling schemes are required for determining a minimum set of selected soil properties and processes that fulfill the requirements for soil quality indicators (i.e. sensitivity, relation to specific soil functions or ecosystem services). A baseline of information that describes the initial soil conditions is the initial step for applying management plans, as it enables identification of management effects over time. Differences between land uses observed in this study indicate the need to define baseline values for each land use. Periodic soil evaluations for monitoring changes and management effects should follow. Ideally, monitoring should go beyond fertility assessments, and include indicators of soil quality such as those suggested in this study.

6.5 General conclusions

Informed soil management decisions that contribute to long-term agricultural production requires understanding the changes in soil quality in response land use and management practices. This study investigated soil quality of three land uses (potato crops, short fallow fields, and eucalyptus plantations) located in mountainous areas of the Central Andean region of Bolivia. The originality of this study relies on the integrative approach for

addressing soil quality, accounting for soil physico-chemical properties, soil aggregates, microbial processes and their interactions. Moreover, to our best knowledge, this study presents the first report of soil quality assessment of agricultural ecosystems in Bolivia and the Central Andes. The study area belongs to a typical agricultural community in a region that encompasses more than 7000 potato farmers. It is thus representative of the agricultural practices conducted by farmers and land-use changes occurred in this region.

We demonstrated that short fallow periods had a neutral effect on soil quality for agricultural production compared to potato crops. This practice did not result in soil fertility restoration or soil structure improvement, as it did not lead to major changes in soil properties, microbial processes, aggregate stability and size distribution compared to fields cultivated with potato. Main effects of this practice were the decrease of labile N content and net N transformation rates indicating the sensitivity of these processes to land-use change and a potential decrease of available N to plants. Fertilization of potato crops with chicken manure was adopted as an alternative practice for managing fertility. This practice improved soil chemical and biological quality (increased labile C and N fractions, soil pH, and respiration potential), which contributed to overall soil quality for agricultural production. Concomitantly, plant development (seed germination, shoot length, and biomass production) improved as a result of chicken manure fertilization. Compost turning frequency and maturity did not influence these soil properties. However, application of manure at early stages of decomposition should be avoided to reduce the risk of phytotoxic effects, since we observed root damage in *A. sativa* seedlings caused by extracts of immature manure. Composting manure at least 100 days and conducting monthly turning events should be incorporated as manure management practice to accelerate decomposition process and ensure proper stabilization and maturation.

The plantation of eucalyptus increased soil physical quality compared to potato cultivated land use (higher megaaggregate content and stability). However, changes in soil properties and processes (reduced metabolic potential of soil bacteria, carbon use efficiency, net N mineralization, and abundance and activity of ammonia oxidizing archaea and bacteria) indicated lower chemical and biological soil quality. Overall, eucalyptus reduced soil quality for agricultural production, and its use for afforestation programs should be avoided in agricultural areas, especially in communities where agriculture constitutes the main economic income.

This study demonstrated that ammonia-oxidizing archaea were dominant nitrifying organisms in terms of abundance and activity under the three land uses. The contribution of AOB to nitrification increased with increasing pH. Agricultural practices leading to increased soil pH and labile N content (e.g. chicken manure fertilization) would thus determine contribution of AOB and AOA to nitrification. This contribution, however, should not be inferred from *amoA* gene abundance alone, but measured separately through process rate studies, to determine the environmental consequences of land -use management.

The labile hot water extractable C and microbial biomass were the good indicators of changes in response to land use and fertilization in soil functions related to C and N

mineralization and soil structure. Moreover, we found evidence that microbial activity contributed to aggregate formation and stability. Changes in these labile C fractions and microbial processes should be considered for monitoring changes in soil functions and soil structure. Also the hot water extractable C, N and respiration potential were sensitive to fertilization of soils with chicken manure, and should be used for monitoring agricultural practices.

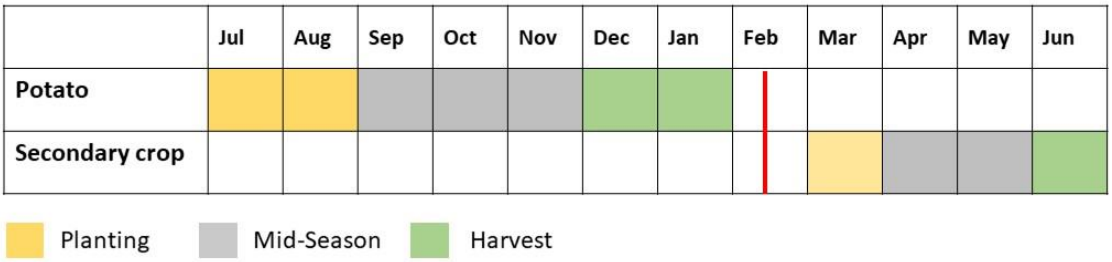
The information obtained in this study allowed a mechanistic understanding of the responses of soils to land-use changes, which is the basis for guiding sustainable soil management practices and for improving agricultural production. Specifically, given that the study area is representative of the agricultural practices and land-use changes occurring in potato-based agricultural ecosystems, this information should be used to guide future management practices, land-use planning, and government policies for the region.

Appendix

Supplementary material

Supplementary material to Chapter 2 "Soil properties and microbial processes in response to land-use change in agricultural highlands of the Central Andes"

S1. Description of a typical rotation cycle. The cycle starts with potato cultivation, followed by a secondary crop (*Vicia faba* L., faba beans; *Hordeum vulgare* L., barley) and the cycle is repeated again or the field is converted to fallow or eucalyptus plantation. The red line indicates the sampling time.



S2. Summary statistics of ANOVA tests conducted on fitted linear simple models (one sample per plot) to test differences of soil physico-chemical variables between land uses (n=8). For each variable the degrees of freedom of the numerator (DF_{num}) and denominator (DF_{den}), factor's mean sum of squares of (Sum Sq), residual's mean sum of (Res Sq) F statistic (F), and P-value, and the model R-square (R^2) are presented. Significant differences are highlighted in bold. WHC: water holding capacity, TOC: total organic carbon, TN: total nitrogen, TP: total phosphorous, Soil C:N: TOC/TN (mass basis).

Variable	DF_{num}	DF_{den}	Mean Sq	Res sq	F	P-value	R^2
Sand	3	21	69.92	65.64	1.06	0.36	0.01
Silt	3	21	43.35	41.55	1.04	0.37	0.00
Clay	3	21	17.98	93.66	17.98	0.19	0.00
WHC	3	21	38.94	31.51	1.24	0.31	0.02

S3. Summary statistics of nested ANOVA tests conducted with mixed-effects models (3 samples per plot, 8 plots per land use; fixed effect: land use, random effect: plot) for soil physico-chemical and microbial variables. For each variable the degrees of freedom of the numerator (DF_{num}) and denominator (DF_{den}) F statistic (F), P-value, and the conditional ($R^2_{LMM(c)}$), marginal ($R^2_{LMM(m)}$), random ($R^2_{LMM(r)}$) and error ($R^2\varepsilon$) R-squares are presented.

Variable	DF_{num}	DF_{den}	F	P-value	$R^2_{LMM(c)}$	$R^2_{LMM(m)}$	$R^2_{LMM(r)}$	$R^2\varepsilon$
pH_{KCl}	3	21	3.90	0.06	0.97	0.28	0.69	0.03
SOM	3	21	235.6	< 0.001	0.89	0.31	0.58	0.11
EBC	3	21	111.8	< 0.001	0.79	0.20	0.59	0.21
Ca^{2+}	3	21	96.21	< 0.001	0.90	0.25	0.65	0.10
Log(Mg^{2+})	3	21	3.07	0.07	0.69	0.16	0.53	0.31
Sqrt(Na^{+})	3	21	116.79	< 0.001	0.68	0.46	0.22	0.32
Sqrt(Fe^{3+})	3	21	240.02	< 0.001	0.48	0.28	0.20	0.52
Log(Mn^{2+})	3	21	500.47	< 0.001	0.73	0.25	0.48	0.27
K^{+}	3	21	53.55	< 0.001	0.80	0.22	0.56	0.20
Log(Al)	3	21	19.9	< 0.001	0.87	0.36	0.50	0.13
Extractable P	3	21	112.5	< 0.001	0.95	0.69	0.27	0.05
Log(HWC)	3	21	4307.8	< 0.001	0.99	0.42	0.57	0.01
HWN	3	21	2.42	0.11	0.87	0.16	0.72	0.13
HWC:HWN ratio	3	21	4902.8	< 0.001	0.90	0.64	0.26	0.10
WSC	3	21	1.07	0.36	0.99	0.09	0.90	0.01
WSN_{tot}	3	21	76.5	< 0.001	0.99	0.71	0.29	0.01
WSN_{org}	3	21	0.77	0.47	0.77	0.05	0.72	0.23
WSC:WSN_{org} ratio	3	21	75.62	<.0001	0.59	0.59	0.00	0.41
MBC	3	21	102.1	< 0.001	0.88	0.45	0.42	0.12
MBN	3	21	100.0	< 0.001	0.83	0.54	0.29	0.17
MBP	3	21	48.1	< 0.001	0.80	0.50	0.29	0.20
MBC:MBN	3	21	141.49	< 0.001	0.81	0.54	0.27	0.19
MBC:MBP	3	21	12.93	< 0.001	0.11	0.03	0.07	0.89
MBN:MBP	3	21	15.55	< 0.001	0.45	0.07	0.38	0.55
AWCD	3	21	150.9	< 0.001	0.74	0.43	0.31	0.26
Log(Respiration)	3	21	217.8	< 0.001	0.90	0.66	0.24	0.10
qmic	3	21	387.2	< 0.001	0.94	0.62	0.32	0.06
qCO_2	3	21	392.8	< 0.001	0.76	0.44	0.32	0.24
Nmin	3	21	302.3	< 0.001	0.93	0.76	0.17	0.21
Net nitrification	3	21	59.7	< 0.001	0.83	0.68	0.15	0.17
Relative nitrification	3	21	203.9	< 0.001	0.98	0.65	0.33	0.02

S4. Correlation table of soil carbon fractions: soil organic matter (SOM), hot water extractable carbon (HWC), water soluble carbon (WSC), microbial biomass carbon (MBC) of soils under potato, fallow and eucalyptus land uses (Pearson correlation coefficient, ** = *p*-value <0.01, n=72).

	SOM	HWC	WSC	MBC
SOM	-			
HWC	0.80**	-		
WSC	0.38**	0.68**	-	
MBC	0.83**	0.92**	0.55**	-

Supplementary material to Chapter 3 "Contribution of soil microbial biomass and oxy-hydroxides to aggregate stability and size distribution under different land uses in the Central Andes"

S1. Results of regression analyses conducted between microbial biomass C (MBC), dithionite-extractable iron (Fe_{DCB}), and oxalate-extractable iron and manganese (Fe_{Ox}, Mn_{Ox}) with the aggregate stability indexes mean weight diameter (MWD), water stable aggregates (WSA) and aggregate sizes. For all relationships the intercept (α), slope (β), and the regression coefficients (R^2) are presented. *= *p*-value <0.05.

	MBC			Fe_{DCB}			Fe_{Ox}			Mn_{Ox}		
	α	β	R^2	α	β	R^2	α	β	R^2	α	β	R^2
MWD	0.64	0.001*	0.49	0.83	0.02*	0.20	1.44	-0.13*	0.15	1.35	-0.49*	0.27
WSA	75.0	0.02*	0.32	80.6	0.29	0.06	99.2	-5.03*	0.28	93.45	-14.27*	0.29
Megaaggregates	-36.6	0.57*	0.57	84.6	8.33*	0.20	349.1	-50.75	0.05	361.42	-257.17*	0.25
Macroaggregates	532.2	-0.25*	0.21	468.2	-2.78	0.02	441.8	-4.48	0.00	328.30	162.74*	0.22
Free Microaggregates	168.6	-0.15*	0.32	155.0	-3.48*	0.33	37.9	23.47	0.12	75.29	49.14	0.05
Free Silt+clay fraction	220.8	-0.21*	0.34	171.1	-2.60	0.07	4.24	45.03*	0.29	50.22	136.14*	0.35

S2. Results of linear regression analyses conducted between potential respiration and net N mineralization with the aggregates quantity in the soil (g kg^{-1}). For all relationships the intercept (α), slope (β), and the regression coefficients (R^2) are presented. *= *p*-value <0.05, **= *p*-value <0.01.

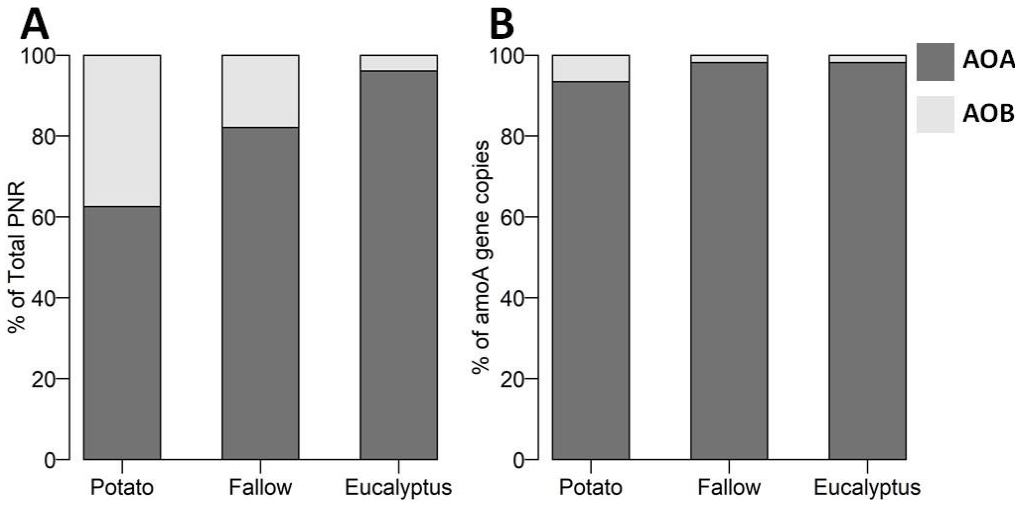
Aggregate size classes	Potential respiration			net N mineralization		
	α	β	R^2	α	β	R^2
Megaaggregates	0.12	0.001**	0.75	1.14	-0.001	0.07
Macroaggregates	0.86	-0.001**	0.27	-0.22	0.002	0.04
Free Microaggregates	0.67	-0.003**	0.48	-0.02	0.007*	0.17
Free Silt+clay	0.64	-0.002**	0.40	0.42	0.003	0.00
Occluded microaggregates	0.66	-0.003**	0.32	0.07	0.007	0.08
Occluded silt+clay	0.54	-0.00	0.04	0.13	0.003	0.03

S3. Results of linear regression analyses conducted between potential respiration and net N mineralization with TOC and TN content of aggregate sizes. For all relationships the intercept (α), slope (β), and the regression coefficients (R^2) are presented. *= *p*-value <0.05, **= *p*-value <0.01.

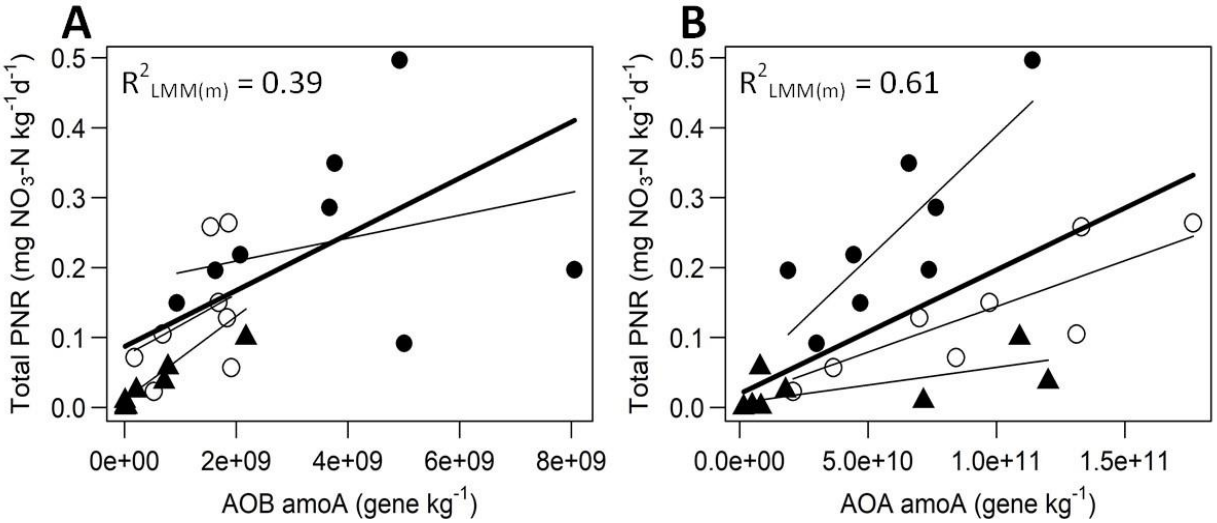
	Aggregate size classes	Potential respiration			net N mineralization		
		α	β	R^2	α	β	R^2
	Megaaggregates	0.20	0.03**	0.77	0.85	-0.01	0.03
	Macroaggregates	0.33	0.00	0.04	-0.24	0.09*	0.14
TOC	Free Microaggregates	0.47	-0.05	0.02	-0.27	0.49**	0.42
(g C kg^{-1} soil)	Free Silt+clay	0.52	-0.05	0.06	0.07	0.21	0.08
	Occluded microaggregates	0.47	-0.04	0.04	0.04	0.30*	0.22
	Occluded Silt+clay	0.15	0.04	0.12	-0.07	0.15	0.10
	Megaaggregates	0.16	0.43**	0.89	0.83	-0.15	0.04
	Macroaggregates	0.41	-0.04	0.04	-0.39	1.19*	0.16
TN	Free Microaggregates	0.52	-0.78	0.12	-0.27	5.27**	0.43
(g N kg^{-1} soil)	Free Silt+clay	0.57	-0.55	0.10	-0.13	2.44*	0.15
	Occluded microaggregates	0.51	-0.66	0.13	0.03	3.31**	0.24
	Occluded Silt+clay	0.18	0.28	0.07	-0.14	1.39*	0.13

Supplementary material to Chapter 4 "Response of ammonia-oxidizing bacteria and archaea abundance and activity to land-use changes in agricultural systems of the Central Andes"

S1 Relative importance of AOB and AOA. Average contribution of AOB and AOA (A) to total potential nitrification rates (PNR), and (B) to total number of *amoA* gene copies in the three land uses.



S2 Relationships of *amoA* gene copy numbers of A: ammonia oxidizing bacteria and B: ammonia oxidizing archaea with total potential nitrification rates (PNR). The thick lines were fitted for all data points (n=24) and thin lines were fitted for each land use separately (n=8), points represent soils under potato (●), fallow (○) and eucalyptus (▲) land use.



S3 Results of the linear mixed effects models of the relationship between total potential nitrification rates and *amoA* gene copy numbers of AOA and AOB in each land use. The intercepts, estimated slopes and regression coefficients R^2 are presented for each land use (* *p*-value <0.05, ** *p*-value <0.01).

Explanatory variable	Land use	Intercept	Slope	R^2
amoA-AOB	Potato	0.18	8.5×10^{-09}	0.00
	Fallow	0.07	6.8×10^{-08}	0.17
	Eucalyptus	0.01	4.6×10^{-08} **	0.94
amoA-AOA	Potato	0.04	3.5×10^{-09} **	0.64
	Fallow	0.01	1.3×10^{-09} **	0.69
	Eucalyptus	0.00	5.1×10^{-10}	0.22

S4 Results of the linear mixed effects models of the relationship between AOA and AOB potential nitrification rates with their respective *amoA* gene copy numbers in each land use. The intercepts, estimated slopes and regression coefficients R^2 are presented for each land use (* *p*-value <0.05, ** *p*-value <0.01).

Explanatory variable	Land use	Intercept	Slope	R^2
amoA-AOB	Potato	0.03	1.8×10^{-08}	0.01
	Fallow	0.001	2.1×10^{-08} *	0.14
	Eucalyptus	0.001	2.1×10^{-08} **	0.86
amoA-AOA	Potato	0.03	1.7×10^{-09} *	0.50
	Fallow	0.02	1.0×10^{-09} **	0.86
	Eucalyptus	0.03	1.1×10^{-10}	0.23

S5 Results of the linear mixed effects models of the relationship between total, AOA and AOB potential nitrification rates (PNR) with soil pH_{KCl} in each land use. The intercepts, estimated slopes and regression coefficients R^2 are presented for each land use (*p-value <0.05, ** p-value <0.01).

		Intercept	Slope	R^2
Total PNR	Potato	-0.41	0.16*	0.14
	Fallow	-0.22	0.09	0.10
	Eucalyptus	0.09	-0.02	0.00
AOB-PNR	Potato	-0.53	0.15*	0.37
	Fallow	-0.17	0.05*	0.16
	Eucalyptus	0.01	0.00	0.00
AOA-PNR	Potato	0.12	0.01	0.00
	Fallow	-0.01	0.03	0.00
	Eucalyptus	0.13	-0.02	0.00

Supplementary material to Chapter 5 "Effects of chicken manure management on soil quality and plant development"

S1. Left: roots without rood damage. Right roots with signs of apparent damage in the form of tissue oranging. Different background color for A and B are presented to improve contrast and distinguish differences.

S2. Chemical composition of the nutritive solution used to water technical control pots containing Fontainebleau sand.

Nutrient	Concentration
Total N	150 mg l^{-1}
Phosphorus	30 mg l^{-1}
Potassium	60 mg l^{-1}
Magnesium	12 mg l^{-1}
Boron	60 µg l^{-1}
Iron	120 µg l^{-1}
Manganese	60 µg l^{-1}
Molybdenum	6 µg l^{-1}
Zinc	12 µg l^{-1}

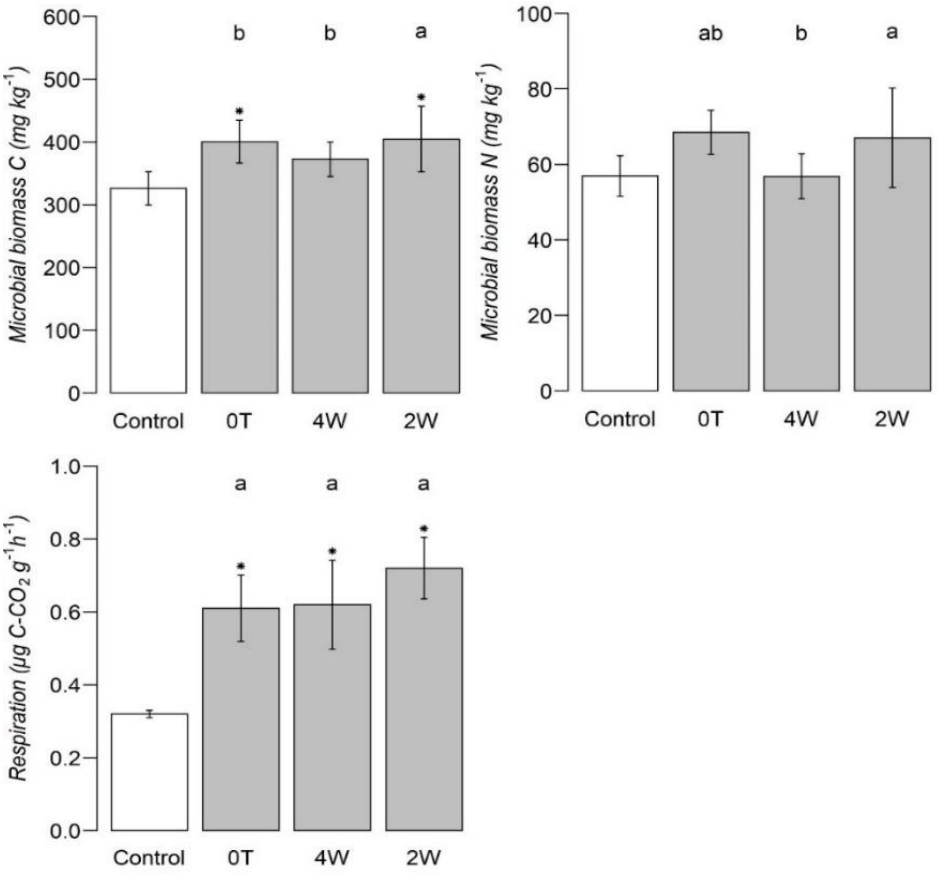
S3. Two-way ANOVA table for the effect of chicken manure extracts and chicken manure fertilization on seed germination, plant development and soil characteristics. Chicken manure used was subjected to three Turning Frequencies Treatments (0T = no turning of the piles; 4W = turning every 4 weeks; 2W = turning every 2 weeks.), and to different composting time (compost age). For each main effect (turning frequency and compost age) and their interaction, the degrees of freedom (df), sum of squares (sum of Sq), the F-value, and *p*-value are presented.

	Effects	df	Sum of Sq	F-value	*p*-value
Seed germination (%)	Turning Frequency	2	2.89	0.35	0.71
manure extract	Compost age	5	18.05	0.88	0.50
	Interaction	10	48.75	1.19	0.33
Apparent root damage (%)	Turning Frequency	2	14.74	21.86	<0.01
	Compost age	5	75.19	44.59	<0.01
	Interaction	10	4.33	1.28	0.28
Seed germination (%) fertilized soil	Turning Frequency	2	3.7	0.01	0.99
	Compost age	5	320.4	0.38	0.85
	Interaction	10	1351.9	0.80	0.63
Root length (cm)	Turning Frequency	2	63.21	1.83	0.17
	Compost age	5	223.81	2.59	<0.05
	Interaction	10	111.17	0.64	0.77
Shoot length (cm)	Turning Frequency	2	37.38	2.33	0.11
	Compost age	5	57.44	1.43	0.23
	Interaction	10	98.31	1.23	0.31
Dry matter (g)	Turning Frequency	2	0.00	2.39	0.11
	Compost age	5	0.01	1.76	0.14
	Interaction	10	0.01	1.75	0.11
pH_{KCl}	Turning Frequency	2	0.28	41.78	<0.01
	Compost age	5	0.08	4.75	<0.01
	Interaction	10	0.31	9.10	<0.01
WSC (g kg^{-1})	Turning Frequency	2	2018.5	4.03	<0.05
	Compost age	5	6326.3	5.05	<0.01
	Interaction	10	6209.5	2.48	<0.05
WSN (g kg^{-1})	Turning Frequency	2	28.10	0.96	0.39
	Compost age	5	158.80	2.17	0.08
	Interaction	10	162.60	1.11	0.38
HWC (g kg^{-1})	Turning Frequency	2	11578	1.15	0.33
	Compost age	5	14834	0.59	0.71
	Interaction	10	69929	0.38	0.23

S3. Continuation

	Effects	df	Sum of Sq	F-value	*p*-value
HWN (g kg^{-1})	Turning Frequency	2	7.55	0.13	0.88
	Compost age	5	75.21	0.50	0.77
	Interaction	10	308.15	1.03	0.44
MBC (mg kg^{-1})	Turning Frequency	2	10904	4.70	<0.05
	Compost age	5	15981	2.75	<0.05
	Interaction	10	21293	1.83	0.08
MBN (mg kg^{-1})	Turning Frequency	2	1449.1	13.13	<0.01
	Compost age	5	1074.0	3.89	<0.01
	Interaction	10	1078.5	1.95	0.07
Respiration potential (µg C-CO_2 h^{-1} g^{-1})	Turning Frequency	2	0.13	6.74	<0.01
	Compost age	5	0.04	0.81	0.55
	Interaction	10	0.11	1.13	0.37

S4. Effects of turning frequencies of manure piles (0T: no turning of manure piles, 4W: turning every 4 weeds, 2W: turning every 2 weeks) on microbial biomass C and N. For each treatment the mean and standard deviation are presented (n=18). Asterisks indicate significant difference in comparison to control not fertilized soil (Dunnett's test, *p*-value <0.05). Different letters indicate significant differences between turning frequency treatments (two-way ANOVA and Tukey tests, *p*-value <0.05).



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